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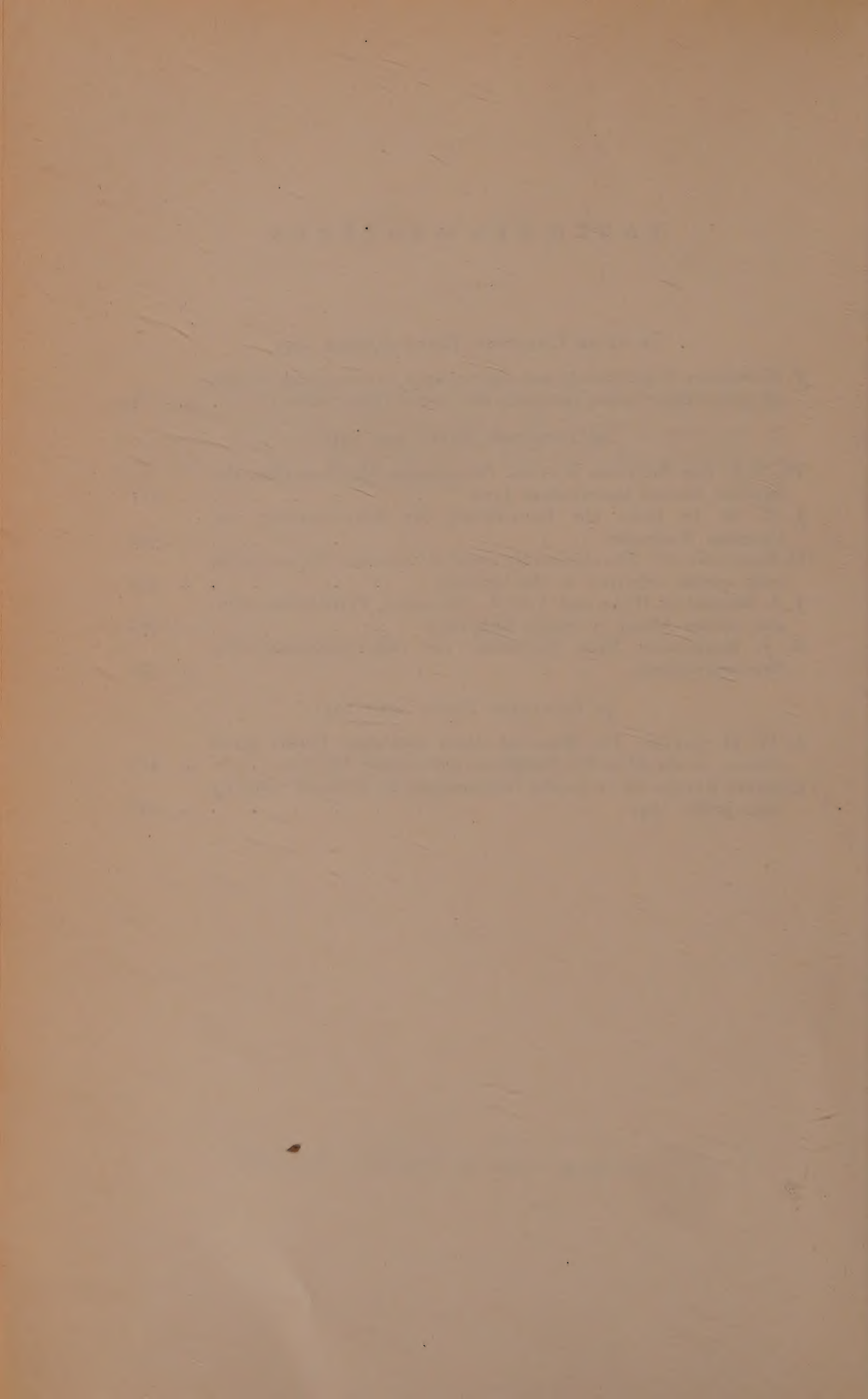sche Dierkundige Vereeniging.

TABLE DES MATIÈRES

EXPERIMENTS AND OBSERVATIONS ON SWARMING, PELAGIC LIFE AND SETTING IN THE EUROPEAN FLAT OYSTER, OSTREA EDULIS L.

BY

P. KORRINGA

CONTENTS

INTRODUCTION

Periodical decline in the productiveness of oyster beds is a world-wide feature. Practically every oyster producing region has not only seen prosperity but also adversity. Overfishing, enemies, unusual mortality and inadequate spatfalls, may rapidly decrease the population of the oyster beds almost to extinction point. At such times, when the future looks very gloomy and uncertain to the oyster farmers, the Government usually will lend a helping hand. Scientific investigations will be made to find the way to restore normal conditions.

A very important change was effected in the second half of the nineteenth century by stopping the free fishery on the natural oyster beds and by beginning a real oyster culture, of

which the principal features are the regulation of the fishery
and the provision of a suitable cultch for the young fry. The
oyster grounds were withdrawn from the free fishery and a
leasing system passed these grounds on to private use. The
declining production rendered these measures necessary and
scientists in many countries recommended the measures to be
taken and urged to take prompt action. France was the first
country to effect this change of paramount importance to a
real oyster culture, which was to result in an unexpected revival
of the oyster industry. About 1860 Professor CostE (1861) was
the champion in France, rediscovering the Italian methods of
spat-collection and applying them on a large scale. The unex-
pected good results were the cause of a rapid change in oyster
culture in many countries of Europe and in America, and there
are but few places in civilised countries nowadays, where the
oyster industry still clings to the old free fishery without some
form of oyster culture.

This change did not solve all problems, for it soon appeared
that a decline of oyster beds was still possible. Thus the oyster
producing regions of France as well as those of England were
up against a very high mortality, which led to an almost complete
exhaustion of the oyster grounds in the year 1921.

Moreover the countries in the northern parts of the range of
the oyster often suffered from inadequate spatfall. Continued
failures of spatfall over several consecutive seasons is disastrous
for oyster culture.

An increase in the production of spat is the best way to get
out of these difficulties. Such an increase can be achieved by
more intensive and more efficient methods of spat-collection,
which require a thorough knowledge of the biology of the oyster
and the oyster larvae and of the demands these organisms make
upon environmental conditions.

In those countries where the natural conditions do not regu-
larly ensure a sufficient spatfall attempts were made to effect
an artificial production in enclosed sea-ponds or in specially
prepared pits. It does not seem very probable that artificial
oyster culture will soon be carried out on a large commercial
scale, for though it proved to be possible, after a great many
vain attempts, thus to produce a profuse settlement, this system
requires so much labour and expense that competition with the
natural system does not seem possible as yet. Importation of

seed oysters from regions where the breeding grounds are more suitable is as yet more economical.

It has been shown that one of the most successful methods to intensify the natural spatcollection is the providing of the cultch at the right time. Clean cultch laid out just at the moment when the mature oyster larvae are on the point of setting ensures a spatfall many times larger than a cultch immersed many days previous to the setting. Already WINSLOW (1884) declared that "thousands of dollars would be saved annualy by the oyster-men if they would determine with any approximate accuracy the date when attachment of the young oysters would occur".

Especially in France, since the crisis in 1921, and in America extensive inquiries have been made into the periodicity in the production of oyster larvae and into the way of forecasting with some accuracy the moment of spatfall, if possible on long term. Each season these investigations are carried out in many impor-tant seed producing centres and bulletins are issued to communi-cate the right time for the planting of cultch and collectors.

The years following 1930 were extremely hard for the oyster farmers in Holland. An extraordinarily rapid propagation of the slipper limpet, *Crepidula fornicata*, on the oyster grounds in Holland, where it has found a very congenial home, made it impossible to continue with the application of *Cardium*-shells as cultch-material. After the tremendous extension in the English oyster grounds, where *Crepidula* was imported with oysters from America in about 1880, the slipper-limpet came to the waters of Holland in about 1925, probably on the sea currents, attached to wreckage or sea-weed. I found a piece of wreckage covered with many large Actinia and about twenty living slippers on the beach near Zandvoort in 1926. The disastrous extension in Holland began about 1930. The slipper did not reach the French oyster regions, as the direction of the prevailing seacurrents safeguard the French shore against such an unwelcome invasion.

The *Cardium*-shells, which were yearly sown out in large quantities on the oyster grounds in Zeeland, proved to be an excellent cultch-material for the young slippers, too, which soon overcrowded the oyster spat. It was necessary to return to the old tile-collector system used in the years about 1885, which requires far more labour and expense than the sowing out of the *Cardium*-shells. These limed tiles are immersed in the water shortly before the spatfall can be expected and by regular

cleaning and by detroquage in the next winter an efficient combat-
ting of undesirable rivals and enemies is made possible. Moreover
a disease caused an enormous mortality during these years
(HAVINGA 1931, KORRINGA 1939) and most of the surviving
oysters were in a bad condition.

The most important measures taken are repeated cleaning
of the oyster grounds in order to keep the slippers within bounds,
the importation of French seedoysters in order to obtain many
oyster larvae as well as grown-up oysters of good quality in the
following winter-season and the prediction of the spatfall by
studying the quantity of oyster larvae in the plankton during
the season of propagation.

In 1935 Dr. HAVINGA (1938, 1939) carried out these studies
for the first time in Holland, using a more perfect and better
quantitative method than the French investigators and moreover
Dr. GRIJNS checked the spatfall, so that afterwards it could be
seen if the forecasts were right.

As the study of the oyster larvae in daily plankton samples
requires a good deal of time and effort, Dr. HAVINGA soon
found it impossible to carry out these investigations in addition
to his other work in the Governmental Institution for Biological
Fishery Research. Since 1936 I have pursued these investigations
every summer, the first year at the laboratory of Dr. HAVINGA
in Amsterdam and after that in the laboratory of the Fishery
Board of the Zeeland Streams at Bergen op Zoom. Moreover
I carried out many investigations on the biology of the oyster
larvae and on the setting behaviour of our native oyster, *Ostrea
edulis*. The results of these studies as well as those of the daily
plankton samples will be discussed in the following sections. As
far as possible the results obtained by other investigators, often
with other kinds of oysters, will be compared with those of
Ostrea edulis.

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me to study the larval stages and the setting process simultaneously.

I wish to express my thanks to Dr. HAVINGA, director of the section Inshore Fishery of the Governmental Institution for Biological Fishery Research for introducing me to the problems of oysterbiology and for his constructive criticism. It is a pleasure to express my thanks to the Fishery Board of the Zeeland Streams for giving me hospitality in their laboratory at Bergen op Zoom and for placing at my disposal their police-vessels in behalf of these investigations. The punctual assistance of commanding officers and crew of these vessels, who often worked at night and in bad weather, has contributed much to the success of these experiments. Finally I wish to thank Mr. BAKKER, the skilful laboratory attendant, for the construction of much apparatus and for the preparation of the material used.

I. THE PROPAGATION OF THE OYSTER

General information concerning the propagation of the different species of the genus *Ostrea* and the state of our knowledge of the morphology and anatomy of the oyster larvae.

It is outside my scope to give here a detailed description of the morphology and anatomy of the reproductive organs of *Ostrea edulis*. HOEK (1884) made an elaborate study of this subject and elucidated his paper with clear figures. So far no better study of the reproductive organs of the native oyster has come to my knowledge.

I consider the protandric alternating hermaphroditism of this kind of oyster to be generally known.

In the reproductive season the sperm and ripe eggs are liberated from the gonaducts of the spawning oyster and are extruded into the suprabranchial chamber (syn. cloacal chamber, syn. exhalent chamber). The sperm leaves this suprabranchial chamber, which is situated right under the adductor muscle and above the junction of the gill-bases, in the same way as the outgoing stream of the water current; so it follows the same course as the waste products from rectum and kidneys.

The ripe eggs do not leave the mother oyster in this simple and easy way. They first pass into the branchial chamber (syn. mantle chamber, syn. inhalent chamber.) Further particulars

about the spawning act will be discussed in the section on spawning. After this passage to the branchial chamber the eggs are either extruded at once into the surrounding sea water or (in other species of oysters) the eggs are held for some time in the branchial chamber adjacent to the gills and labial palps. Here they then develop for a considerable period before they leave the mother oyster. Such species of oysters are often called viviparous, though it would be better to call them incubatory or embryophorous. This difference in the time during which the larvae are held in the branchial chamber is very important. Though this study deals with *Ostrea edulis*, an embryophorous species, many remarks will be made about other kinds of oysters, for many papers on those oysters will be discussed. Therefore I herewith give a table of the two reproductive types of oysters, only including species of economic value.

Incubatory and larviparous	*Non-incubatory, spawning directly into the seawater*
Ostrea edulis L, Huître plate European flat oyster.	*Ostrea virginica* Gmelin American oyster, Bleu-Point.
Ostrea lurida Carpentier Olympia Oyster, British Columbian Oyster.	*Gryphaea angulata* Lamarck Portuguese Oyster.
Ostrea denselamellosa Lischke Japanese Oyster.	*Ostrea gigas* Thunberg Common Japanese Oyster.
Ostrea Angasi Sowerby Australian mud-oyster.	*Ostrea commercialis* Iredale and Roughley Australian rock-oyster.
Ostrea lutaria Hutton New Zealand Oyster.	*Ostrea cuculata* Born Indian Oyster.

It is not very certain that all these forms are really different species. Oysters are difficult subjects to the systematist, as the shell-shapes are to a high degree dependent on environmental conditions.

For the greater part the studies discussed deal with two incubatory species of oysters, viz. *Ostrea edulis* and *Ostrea lurida*, and moreover with *Ostrea virginica* and *Ostrea gigas* as non-incubatory species.

The process by which the reproductive elements leave the gonaducts is always called the spawning act. The final release of the larvae from the material brood-chamber in larviparous

species of oysters is called the liberation of the larvae or swarming
act. The term swarming was first used in oyster literature by
STAFFORD (1914) in contrast with the original spawning whereby
the eggs are released from the gonaducts. We may say that
swarming is only a delayed completion of the spawning process.

In both incubatory and non- incubatory types of oysters the
eggs are finally discharged from the branchial chamber.

Many scientists have studied the morphology and anatomy of
the oyster larvae:

Ostrea edulis.

BRACH (1689) was the first to observe the larvae of *Ostrea
edulis*, the European oyster, microscopically. He found the eggs
as a white liquid mass in the branchial chamber of oysters near
Venice during the summer months and established the fact
that pigmented shellbearing larvae can be detected at the time
this mass has turned dark-coloured and pappy. LEEUWENHOEK
(1722), independently of BRACH's study, found swimming
larvae in the maternal broodchamber. He also noticed young
less motile embryos and discovered the sperm of the oyster.
DAVAINE (1853) gave the first detailed description of the develop-
ment of the oyster larva, starting from the four-blastomeric
stage. His description of the shedding of the velum during meta-
morphosis is contradicted by COLE (1938a). LACAZE–DUTHIERS
(1855) gave some information about anatomical details concern-
ing the intestinal tract, and in the papers of MOEBIUS (1877,
1883) a few figures about the very first stages of larval
development can be found.

It was HORST (1884) who made elaborate inquiries into the
stages of development of the oyster embryo up to that of
swarming. As HORST did not succeed in finding oyster larvae
in the plankton of the Oosterschelde (Holland), neither the
morphology and anatomy of the pelagic larvae, nor the meta-
morphosis into the adult structure during the setting stage could
be made the subject of his study.

DANTAN (1916) described further details of the anatomy of
the oyster larvae and HAGMEIER (1916) amplified these studies
with a short description of the anatomy of older pelagic larvae,
which were obtained during his researches into the possibility
of "artificial" propagation. YONGE (1926) included the larvae

in his study about the structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. He gives a good description of the feeding and of the alimentary organs of the young larva of the European oyster. Moreover he described the anatomy of these organs in a young spat of 1,2 mm shell-length (about 10 days after setting). He did not have at his disposal any inter-mediate stages, so he could not give a description of the me-tamorphosis from larval into adult structure.

This gap in our knowledge has been filled up quite recently by ERDMANN and COLE. ERDMANN (1934) made a fine study of the development and of the anatomy of mature oyster larvae which are about to set. In his paper many fine and detailed figures of serial sections of the larvae and some reconstructions of the entire animal are to be found. He gives a detailed description of the various organs at this stage. I will return to his conception of the function of the so-called "eye" in the section on the setting process.

Finally COLE (1937, 1938 a) gave a description of the meta-morphosis of the larvae to the adult structure during the setting stage and of the fate of the larval organs. So we know a good deal about the morphology and anatomy of the larvae of *Ostrea edulis*.

There is one thing that has struck me and which I did not find in any of these writers. I have seen tens of thousands of oyster larvae in the course of my investigations and I noticed a marked variation in colour and pigmentation. Many writers studied the larvae taken from the mantle chamber of an adult oyster. They did not notice the variation because the larvae of one mother-oyster are all alike in colour and pigmentation. Moreover only living larvae have a natural colour. The charac-teristic orange-red colour of the pelagic oyster larvae may vary from very pale yellowish-green to yellowish-orange, orange-red, and pale crimson to copper-red and purplish-red. The charac-teristic dark pigmentation of the larvae may vary in intensity as well in extent to a very high degree. The innate pigmentation of mantle-edge and shell in adult oysters is likewise subject to a marked variation, probably independent of environmental conditions. Perhaps we may think of these pigmentations as an indifferent hereditary factor, not subject to the eliminating power of the struggle for life and consequently capable of capricious variation.

Ostrea virginica.

Our knowledge about the morphological and anatomical features of the larvae of *Ostrea virginica* at various stages of development was completed till it formed a whole by itself at an earlier date than the knowledge of these features in the larvae of *Ostrea edulis*. After the descriptions of the main points of larval development by BROOKS (1880), RYDER (1882, 1884) and JACKSON (1888) other scientists elaborated these studies. A fine paper on the development of *Ostrea virginica* is that by STAFFORD (1913) in which he gives a full account of the anatomy of both fully developed larvae and recently settled spat. In an earlier paper (1910) STAFFORD described for the first time the appearance of the foot in mature oyster larvae. WELLS (1927) gives a description of the alimentary organs of the larva of *Ostrea virginica*. NELSON (1924 c, 1926) reports the possibility of metamorphosis without attachment in the larvae of the oyster and of the mussel. He suspended mature oyster larvae in a cradle of bolting cloth close to the surface of an aquarium. A few of these larvae showed metamorphosis, with loss of velum and foot and development of the dissoconch shell, though into an unusual shape. Such a metamorphosis without attachment may occur, though rarely, even in nature, as has been shown in two instances in which such unique pelagic dissoconchs, "floating spat", have been found in plankton catches in Barnegat Bay. (New Jersey, U.S.A.)

I have never met with such floating spat in plankton samples containing larvae of *Ostrea edulis*.

There is still difference of opinion concerning the nature and function of the so-called pigment spot occurring in mature larvae. NELSON (1926) advocates the photosensitive character of these pigment spots which corresponds with the opinion of ERDMANN (1934) about the nature of the pigment spot ("eye") in the mature larvae of *Ostrea edulis*. PRYTHERCH (1934 a), on the contrary, denies those pigmentspots a light-sensitive character, but conceives them as leucocyte-generating tissues, from which cells migrate to the blood-stream during metamorphosis. The discussion of this controversy will be continued in the section on the influence of light on the setting process.

Less extensive is the literature on the morphology and anatomy of the larvae of other species of oysters.

Ostrea lurida.

The general embryology of *Ostrea lurida* (Carpenter) and incubatory species has been described well by STAFFORD (1914) and is essentially the same as in *Ostrea virginica*.

HORI (1933) was able to rear the larvae of *Ostrea lurida* to maturity in vitro and setting under artificial conditions was successfully accomplished. Some figures of the larvae of *Ostrea lurida* can be found in this paper of HORI.

Ostrea gigas.

FUJITA (1929, 1934) provides us with a description of the larval development of the Japanese oyster, *Ostrea gigas* Thunberg. YOKOTA (1936) mentions his studies on the development of the various organs of this oyster, but these studies have not been published so far.

A description of the larval development of *Ostrea denselamellosa*, an incubatory species, closely related to *Ostrea edulis*, is to be found in the paper of SENÔ (1929), while some remarks about another incubatory species, *Ostrea angasi*, the larvae of which likewise show a close resemblance to those of *Ostrea edulis*, are made by ROUGHLEY (1925). In another paper ROUGHLEY (1933) gives a description of the larvae of the non-incubatory species *Ostrea commercialis* and deals briefly with the anatomical reorganisation at fixation in this species.

As far as the latter species is concerned, these processes are in general so similiar to those described by STAFFORD for *Ostrea virginica* that ROUGHLEY did not consider it necessary to give a detailed description.

II. THE RANGE OF OSTREA EDULIS

General demands on environmental conditions to obtain an adequate propagation.

Though *Ostrea edulis* can be found from 66° N. lat. in Norway as far as in the Mediterranean, the regions where nature makes a really intensive and extensive oyster culture possible are limited.

The occurrence of oysters in a certain place does not imply the possibility of oyster farming in that locality. It only indicates

that the oysters can keep up the struggle for life in that place, but the equilibrium with the natural conditions may be so unstable that a very slight interference on the part of man may be the cause of a rapid decline of such natural oyster banks. "It is the last straw that breaks the camel's back" is a very appropriate remark in this connection. An intensive oyster culture is only possible in those regions where a sufficient yearly seed-production is assured. The demands on the natural conditions for a successful propagation are fairly high and cannot be satisfied in the northern parts of Europe. Practice and science (ORTON, 1929 a, 1929 b, 1937 a) prescribe the following properties as essential to producing grounds:

A. Waters sufficiently enclosed, if possible with a relatively narrow communication with the open waters. Only such bodies of water ensure an adequate retention of the larvae within the environs of the locality during the free-swimming stage and counteract the dispersing action of the sea-currents on the larval herds.

B. A local seasonal temperature-range giving frequent probabilities of a maximum temperature in the bulk of the sea-water above 18° C, preferably above 20° C for some time. There is a fair margin for variations in salinity.

C. A suitable subsoil, not too soft mud, no moving sands, but preferably a hard sandy mud or muddy shell-gravel. Peat-soil is considered to be very suitable, too.

D. Some other factors, which are to a certain degree under human control, such as a sufficient stock of larvae-producing oysters, the provision of a suitable cultch-material and the combating of enemies.

The most important producing centres of the European oyster are: in France the region called le Morbihan, especially the rivers Auray-le-Bono, Saint-Philibert and Crach and in Holland the Oosterschelde. A good description of the nature, character and history of the French oyster beds can be found in papers by HINARD and LAMBERT (1928) and by LAMBERT (1935, 1936, 1938). A very suitable locality in France is moreover the Basin of Arcachon, where formerly oyster farming used to take place on a large scale. Another kind of oyster, however, *Gryphaea angulata* Lam., has ousted *Ostrea edulis* here to a considerable

extent. In recent years *Ostrea edulis* has been gaining ground in the basin of Arcachon (BORDE and BORDE 1938). Of less importance are the breeding-grounds in the Mediterranean, in Norway and in England.

That temperature is a very important factor was already known to HERDMAN (1893), who stated that the watertemperature in the oyster producing regions in France exceeded 21° C in the open waters (Arcachon). He added: "However, it may be hoped that, although temperatures like this may be favourable, they are not necessary for successful oyster breeding".

Later it appeared that water-temperatures above 20° are very favourable indeed for a profuse settlement of the spat, though some propagation is possible at any temperature above 16° C. A comparison of the results obtained under different temperature conditions is very instructive.

The duration of the season of propagation is dependent on the space of time that the water-temperature is above a certain level. Though production of larvae can take place at temperatures of 15 to 16° C, a successful spatfall can only be expected when the water-temperature rises above 18° C, the more the better. So it goes without saying that the propagation is distributed over a much longer period in the Mediterranean than in the Northern parts of the oyster range.

BRACH (1689) told us that the oysters in the Adriatic Sea near Venice carried larvae in the mantle cavity all summer and in the beginning of autumn. In the Mare Piccole in the Gulf of Tarent in Italy the oysters breed from April to October (ORTON, 1920). MAZZARELLI (1924) informs us that reproduction takes place from March till in August in the lake of Fusaro near Naples. It is remarkable that, though the water-temperatures are very favourable in the Mediterranean, oyster culture never assumed such proportions here as on the West-coast of France. Various other conditions are less favourable in the Mediterranean, such as the nature of the sub-soil, which is often steep and rocky, consequently unsuitable for dredging operations, while perhaps quality and quantity of food-organisms differ to a high degree from those in the Atlantic coastal waters. The higher salinity is not responsible for checking the oyster industry in the Mediterranean.

The farther we go northward the shorter the season of propagation will be, till we reach at last the northern boundary of the

range of *Ostrea edulis*, where propagation is exceptional, alternating with many years without spatfall. It will be understood that no effective oyster culture based on the natural spatfall will ever be possible in these regions. Only localities with very favourable hydrographic conditions ensure an adequate spatfall in the countries around the North Sea. There is but one place that has appeared to be an excellent seed-producing locality on the North-Sea coast and that is the Oosterschelde in Holland.

The estuaries of the English coast are not sufficiently enclosed to ensure a statisfactory retention of the free-swimming larvae and the water-temperature usually does not rise enough to counteract this disadvantage to a sufficient extent. In my opinion only a very abundant production of larvae can bring about a satisfactory spatfall in the English waters. A considerable decrease in the number of adult oysters, especially owing to the mortality of 1921, has since then made such an abundant production of larvae impossible there. Though it is not impossible that the Fall Estuary can produce a moderate amount of seed-oysters when science and practice join in a great effort (ORTON, 1927 a), it is not likely that the English coast will ever be reckoned among the very important seed-producing grounds.

The reason why the Oosterschelde may be considered as the most important centre farthest North for the production of seed-oysters will be discussed in a separate section on the hydrographical conditions of this water.

The German oyster grounds in the Wattenmeer, the "fiskalische Austernbänke", cannot ensure a profuse yearly spatfall, because the water-temperature does not rise enough and the sea-currents disperse the planktonic larvae to a high degree.

Though the Limfjord in Denmark may be considered as a nicely enclosed water, the average summer temperature is too low here. The temperature does not only affect the spatfall in a direct way, but also in a more indirect way, as oysters become mature at a far older age in colder waters than in warmer, which accounts for a far smaller percentage of larvae-producing oysters in such colder waters (SPÄRCK 1925). Although some spatfall can be found even in the colder years, the oyster culture in these regions is dependent on the importation of seed-oysters from other localities. Adult oysters are found to thrive in those colder waters. Though the rate of feeding is correlative to temperature, the checking of growth and fattening in the

summer-months in behalf of the production of the elements of propagation, is much shorter or may even be absent in colder waters. To a certain extent one may say that the colder the water in the summer months, the better the quality of the oysters and the worse the spatfall.

It will be understood that especially countries which are not so fortunate as to possess rich seed-producing grounds, but which do possess excellent localities for growth and fattening, have a great interest in the possibilities of obtaining a sufficient spatfall in enclosed sea-ponds or oyster pits. Many attempts have been made in England (DODGSON, COLE 1936, 1938 b, 1939, BRUCE and PARKE, 1935–1938), Germany, especially at Heligoland (HAGMEIER, 1916, ERDMANN, 1933) and Denmark (SPÄRCK, 1927). Recently it proved to be possible to ensure an adequate spatfall in limited quantities of water, even in glass-houses (Heligoland), but it requires far too much labour and expense to make large scale operations on a commercial basis practical as yet. Though I shall not describe all these attempts, I shall frequently discuss data on the biology of the oyster larvae obtained in the course of these investigations. From a scientific point of view I consider these attempts highly interesting, for they offer the possibility of entering the new and vast, hitherto unexplored field of the heredity of the oyster, which may yield important practical results in the future.

In Norway a self-reliant oyster culture exists, though on a relatively small scale, which produces its seed-oysters in the so-called pollen. These pollen are pools, a few hundred meters wide and 4 to 8 meters deep, which can be completely closed off by a temporary damming-up of the communication with the fjord-water. Their suitability for oyster culture was discovered about 1880 (RASCH, 1880 a, b), but the reason why a profuse spatfall is possible so far north was discovered much later. There are but a few suitable pollen in Norway, so that seed-production cannot be extended very much. The first investigators ascribed the high water-temperatures in these pollen to the nature of the bottom, which consists of a soft black mud. Later it appeared that the pollen present very peculiar hydrographical conditions. There are no important currents, so stratification is possible. The upper layers of the water are fresh or brackish, flowing in from little brooks, while the lower layers consist of the salt fjord water. These fresh upper layers make the vertical exchange

of warmth impossible, as the warm salt water remains heavier
than the colder fresh upper layers. The fresh water acts like the glass
of a glass-house by transmitting a good deal of the sunrays and
by functioning as a cover during the night (HELLAND-HANSEN,
1908, GAARDER and SPÄRCK, 1932, GAARDER, 1933, GAARDER
and BJERKAN, 1934, GAARDER, 1938). The temperature in the
salt lower layer may rise to 25 to 30° C during a short period
in summer. GAARDER (1933) is right when he states: "Das
Pollwasser bildet somit eine kleine südlandische Welt für sich".

In respect to the duration of the season of propagation in the
different localities of the range, the oyster species in America
(*Ostrea virginica* and *Ostrea lurida*) do not differ essentially from
Ostrea edulis.

Ostrea virginica can be found on the East-coast of North America
from Cape Cod to the Rio Grande. *Ostrea virginica* makes high
demands on the breeding-temperature (20° C at least) and the
spawning season here too is correlative with the duration of
such higher temperatures.

While in the South (Texas) spawning oysters occur from
April till in October and the greater part of the setting-season
extends between May 1 and October 1 (MOORE, 1907, 1915;
HOPKINS, 1931), the propagation in the extreme north of the
range takes place during a few weeks in July or August or fails
to take place at all (NELSON, 1928 c). In the North one generally
finds a slow growth, an unreliable reproduction, but a better
quality of the oyster meat. The inner parts of the bays contain
the warmest water here, and are therefore the most suitable
for reproduction, though the subsoil is relatively soft. The
mouths of the bays are generally colder, so less suitable for
seed production, but have a better subsoil and are more suitable
for the cultivation of a superior quality. The farther north,
the more a well conducted culture is indispensable.

All this holds good for *Ostrea lurida*, too, which is cultivated
on the West-coast of North America. The breeding temperature
of this species lies above the 16° level. In the North, in British
Columbia, propagation takes place in July and August (STAFFORD
1913). In the main culture centre, the Puget Sound, setting
occurs during the months of June, July and August (HOPKINS,
1937), while in South-California (La Jolla) this species propa-
gates during seven months, as long as the water-temperature
remains above 16° C (COE 1932 b).
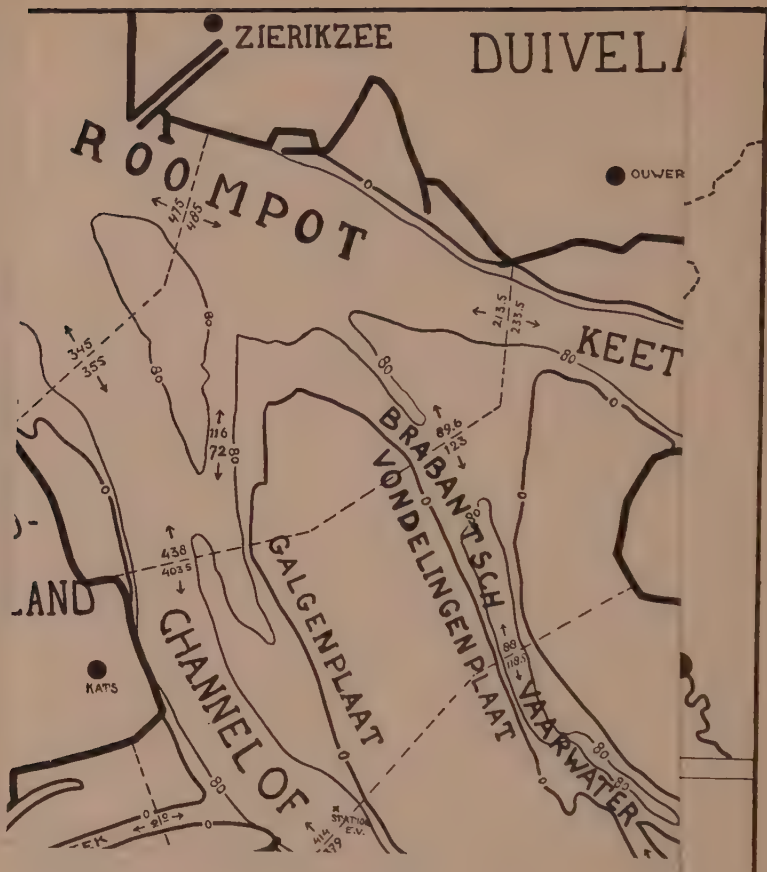
That the young oyster reaches maturity at an earlier age as it lives farther southwards is a rule that applies to *Ostrea lurida* as well (COE, 1931).

NELSON (1928 c) very rightly states, considering the dependence of the duration of the spawning-season on temperature, that these rules hold good for all parts of the range, with no adjustment to the extremes of its distribution. No such adjustment to the environmental conditions has taken place, which a priori was not to be expected either.

III. HYDROGRAPHICAL CONDITIONS

General Description of the Region

The production of oyster brood in the Dutch waters takes place practically exclusively in the Oosterschelde. The Oosterschelde, a bay which penetrates far into the land, is situated in the South-West of Holland, in the province of Zeeland. Centuries ago it was the estuary of the river the Schelde. Gradually a new breaking, the Westerschelde, gained in importance. Finally, in 1869, the communication with the river the Schelde was entirely cut off by the construction of a railway-dam between the mainland and the island of Zuid-Beveland. Since then an other railway-dam, between the islands of Walcheren and Zuid-Beveland, has moreover completely closed off the fresher water of the Westerschelde from the Oosterschelde.

As far as oyster culture is concerned, the eastern part of the Oosterschelde, the bag-shaped widening, bounded on the West by the narrowing between Gorishoek and Kijk-uit (fig. 1) is the most important region. By far the greater part of the oyster beds are situated in this basin, which will be called henceforth in this paper the basin of the Oosterschelde.

An important part of the basin of the Oosterschelde belonged to the inhabited land of Zeeland but some hundreds of years ago. In the beginning of the 16th, partly only in the 17th century, this land was flooded in consequence of dike-bursts. Many local denominations still remind us of those times.

The amount of fresh water that flows into the Oosterschelde is insignificant. Since the communication with the Westerschelde was cut off in 1869, no stream of any importance discharges into the Oosterschelde. Only a few little brooklets from

the mainland and some drained-off water from some polders in the Zeeland islands enter the Oosterschelde. Westward the Ooster- schelde is continuous with the Northsea. Between the islands of Tholen and Duiveland we find the Keeten, a communication with the Grevelingen. In the Grevelingen fairly important variations in salinity occur, caused by the admixture of fresh water coming from the great rivers (the Rhine and the Meuse). Especially when these rivers discharge extraordinarily big volumes of water low salinities will be stated in the Grevelingen. As may be seen on the chart (fig. 1), a greater volume of water moves north-eastwards than south-westwards through the Keeten during a tidal cycle, or in other words: the Keeten shows a surplus of flow. Consequently variations in salinity in the Grevelingen will have little influence upon the salinity in the Oosterschelde.

These properties of the Oosterschelde offer many advantages: no variations in salinity dangerous to oyster culture will ever occur here.

Much of the water that flows away westwards from the basin of the Oosterschelde during ebb, will return to the basin during the next flow. This oscillating movement is possible since no inflowing fresh water regularly forces a considerable portion of this body of water westwards to the Northsea. This oscillating movement prevents a rapid dispersion of the planktonic oyster larvae by the tidal movements and makes possible a rapid warming-up of the water in the basin during fine weather. Especially warmth-absorption by the vast tidal lands is helpful in effecting a rapid rise of the water-temperature. The reverse, a rapid falling of the water-temperature during cold weather, obviously also occurs here.

In cold winters drift-ice will be formed fairly soon in the basin of the Oosterschelde. In consequence of the force of the tidal movements the water will never be frozen over completely here, however.

The bottom configuration of the basin of the Oosterschelde is rather irregular and complicated, as may be seen from the chart (fig. 1). There are deep creeks and channels, often deeper than eight metres (e.g. the Channel of the Oosterschelde and the Lodijksche Gat), and vast tidal lands. These tidal lands consist partly of sand-banks, not suitable for oyster culture, partly of a rather hard sandy mud. The oyster beds are situated mainly on a subsoil of such sandy mud and of peat-soil, which

is found in many places, reminding us of the times that there was land or in any case fresh-water marshland in those places.

Tidal movements

By courtesy of Rijkswaterstaat[1]) I am able to give some information about the tidal movements and the water-currents in the Oosterschelde. Ir. P. Ph. JANSEN was so kind as to submit to me for inspection the data of his hydrographic measurements and to compute for me the degree of water-renewal during a tidal cycle. For this I wish to express my thanks.

Moreover data on observations with the aid of floats for the sake of investigating the direction and the speed of the local current swill be found in the reports by FOKKER (1905) and by HUBRECHT (1884).

The range of the tide in the basin of the Oosterschelde varies between 3 and 4 metres. During an average low water the basin of the Oosterschelde (western boundary Gorishoek-Kijk-uit) contains 275 000 000 cubic metres of water. During an average high water the basin contains 675 000 000 cubic metres of water. Consequently an amount of 400 000 000 cubic metres of water regularly passes the narrowing Gorishoek-Kijk-uit during each ebb, to return for the greater part during the next flow.

These enormous tidal movements, attended with strong tidal currents, cause such an intensive mixing of the water that any permanent stratification in this body of water is thereby prevented. In fact we shall never find a difference in salinity or water-temperature of any importance at different depths in the Oosterschelde that is maintained there till the next tide. The tidal mixing of the water in the basin is thorough, which makes it easier to study the biology of the oyster larvae here than in places where marked stratifications complicate hydrographical conditions. The Eendracht, a shallow water between the mainland and the island of Tholen may be left out of account, as the relatively small volume of water it contains may be considered to perform a continuously oscillating movement. Consequently all the tidal water passes the narrowing Gorishoek-Kijk-uit.

It is very important to know how much of the water passing this narrowing during each ebb, re-enters at the next flow. West

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[1]) Ministry of Public Works, Department for the maintenance of dikes, roads, bridges and the navigability of canals and rivers.

of the basin (fig. 1) we can distinguish the "area of the conduct-
ing channels", of the Brabantsch Vaarwater and the Channel
of the Oosterschelde, both of which conduct the waterstreams
to and from the basin. The Zandkreek, a water situated between
the islands of Noord-Beveland and Zuid-Beveland, may be left
out of account as its content performs an oscillating movement
during a tidal cycle.

The area situated northwest of these conducting channels
may be distinguished as the "outlying district". The volumes
of water which pass through the Brabantsch Vaarwater and the
Channel of the Oosterschelde are not the same during ebb and
flow. In the Brabantsch Vaarwater we shall find more water
passing during flow than during ebb. In several places on the
hydrographical chart (fig. 1) we shall see two numbers, accom-
panied by little arrows, all of them situated in the centres of the
channels. These numbers indicate the volume of water which
passes these channels during one average flow and during one
average ebb, expressed in millions of cubic metres, the left
indicating the flow. A difference between two such numbers
indicates a surplus of flow or a surplus of ebb in that channel.

On the chart it may be seen that the Brabantsch Vaarwater
shows a surplus of flow of 30 000 000 cubic metres. The Channel
of the Oosterschelde consequently shows a surplus of ebb of
30 000 000 cubic metres.

So 30 000 000 m³ of "new" water are conducted via the Bra-
bantsch Vaarwater in south-eastern direction during each tide
(12h 25m). According to Rijkswaterstaat we may speak in this
case of really "new" water, because it is derived from the area
called "outlying district" (the Roompot). Of course the water in
this "outlying district" contains a fraction of the water origi-
nally derived from the basin, brought there in consequence
of the surplus of ebb in the Channel of the Oosterschelde. This
basin-water, however, is considered to be diluted with "new"
water to such a high degree on reaching the Roompot, that
its relative value may be regarded as negligible. Consequently
oyster larvae originating from the basin will be practically
absent in the "new" water of which the surplus of flow of the
Brabantsch Vaarwater consists. We are not justified, however,
in deducing from the foregoing that the tidal renewal of the
water in the basin of the Oosterschelde amounts to 30 000 000 m³.

The Brabantsch Vaarwater discharges itself into the eastern

part of the Channel of the Oosterschelde and not directly into the basin. Consequently a certain part of the 30 000 000 m³ surplus of flow does not reach the basin at all, but streams back westwards via the Channel of the Oosterschelde. According to Rijkswaterstaat the average tidal renewal of the water of the basin may be estimated at about 25 000 000 cubic metres. The different phases of this tidal renewal are sketchily represented in the series of small charts. (fig. 2).

Unlike the watermovements in the "conducting channels",
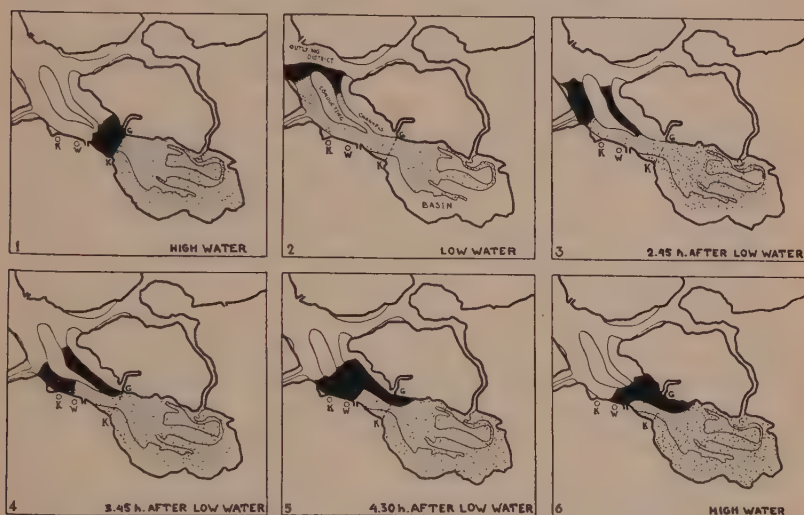
Fig. 2.   Tidal movements.

where the greater part of the waterparticles follow the direction of these channels, the streams in the basin itself are not survey-able in consequence of the irregularity and complexity of its bottom-configuration.

It is certainly possible that a waterparticle, which reaches the basin only near the end of the flow, does not leave the basin during the beginning of the ebb. According to Rijkswaterstaat it is even admissible to assume that all the waterparticles which reach the basin during flow have an equal chance of reaching a certain point after a couple of days. In other words, each waterparticle in the basin, irrespective of its whereabouts in this basin, has an equal chance of remaining in the basin during a couple of days, at least if we do not consider too short a period.

Consequently the 25 000 000 cubic metres of "new" water, which reach the basin at each tide may be considered to drive out from the basin an equal volume of 25 000 000 m³ of the water present in the basin during the preceding tide.

This water streams away in north-western direction, via the Channel of the Oosterschelde (which shows a surplus of ebb) and after this, much diluted, in north-eastern direction via the Keeten, which shows a surplus of flow.

So the water originating from the basin never reaches the western part of the Oosterschelde (West of Zierikzee), but takes its way via the Keeten to the Grevelingen. Strong winds from any direction are considered to have no appreciable influence on the degree to which the water is renewed in the basin. If the period under consideration is not too short, and it is not too short considering the duration of the pelagic stage of the oyster larvae, the influence of the differences between springtides and neap tides on the degree to which the water of the basin is renewed may be considered to be of no importance either.

The foregoing is of paramount importance with regard to the oyster larvae in the Oosterschelde. In the first place it will be clear, assuming that by far the greater part of the oyster larvae are set free on the oyster beds in the basin of the Oosterschelde, that it is of little importance to know during which part of the tidal cycle the larvae are liberated. Even in case of a swarming during ebb, practically all these larvae will return to the basin again during the next flow. Then they start their game of chance: each tide a greater part of these larvae leave the basin and a certain percentage of them never return.

At an average high water the basin contains 675 000 000 m³ of water. Each tide approximately 25 000 000 m³ of this water are renewed, so about 96,3 % of the original water returns.

After two tides $(0,963)^2 \times 675\ 000\ 000$ m³ return and after n tides $(0,963)^n \times 675\ 000\ 000$ m³.

It may be computed that for instance after 14 days less than about 35 % of the original water at high tide is still present in the basin. The rest has streamed away via the Channel of the Oosterschelde and the Keeten, carrying many oyster larvae along with it. The longer the pelagic period, the smaller will be the percentage of the number of larvae originally present in the basin that will remain there till the mature stage. In the section on the horizontal movements of the

larvae we shall see to what degree the movements of the larval herds correspond with the tidal movements.

As will be discussed later on, there are several factors which cause a loss of larvae during their pelagic life. It is often difficult or even impossible to estimate the relative importance of these factors. So it is an advantage that we do know the degree to which the water in the basin is renewed, this being one of these factors.

Apart from the degree of water renewal in the basin during a tidal cycle, there is another factor brought about by the tidal movements, which is often considered of importance with regard to the biology of the oyster larvae. This factor is the velocity of the currents.

In the section on the vertical movements of the larvae and in that on the influence of the currents on the setting process further particulars concerning this factor will be discussed. Rijkswaterstaat investigated the direction and the velocity of the tidal currents during entire tidal cycles at several stations in this region. These stations are situated in the channels as well as in shallower places. Observations were carried out in the surface layers as well as 10 cm and 60 cm above the bottom. It is outside my scope to reproduce here all these data by Rijkswaterstaat.

An important station in connection with my experiments on the setting process is a fairly shallow place on the Yersche Bank called "the Bol" (fig. 1). During low water the depth is 1,50 to 2 metres here, during high water some 3 to 4 metres more. The velocity of the tidal currents at the surface exceeds a maximum of 30cm/sec. here, but does not reach 50 cm/sec. Near the bottom the velocities are about one third less. Another important station is, for instance, the eastern part of the deep Channel of the Oosterschelde near Gorishoek (fig. 1). The velocity of the current in the surface layers in the middle of this Channel reaches a maximum of about 150 cm/sec., the maximum velocity near the bottom exceeding 100 cm/sec.

The station situated in the middle of the Channel of the Oosterschelde near Wemeldinge yields the same figures. In general we may state that in the surface layers of the deep channels maximum current velocities of 100 to 150 cm/sec. may be expected, while the maximum velocities near the bottom are about one third less. The channels within the basin show maximum current-

velocities of 50 to 100 cm/sec., while maximum current-velocities
below 50 cm/sec. will only be found in shallow places.

In correlation with the irregular bottom-configuration in the
basin, directions and velocities of the tidal currents often show
a less regular course in shallow places than in the deep channels.

Salinity and water-temperature

Since March 1921 the commanding officer aboard one of the
policeboats of the Fishery Board of the Zeeland Streams has
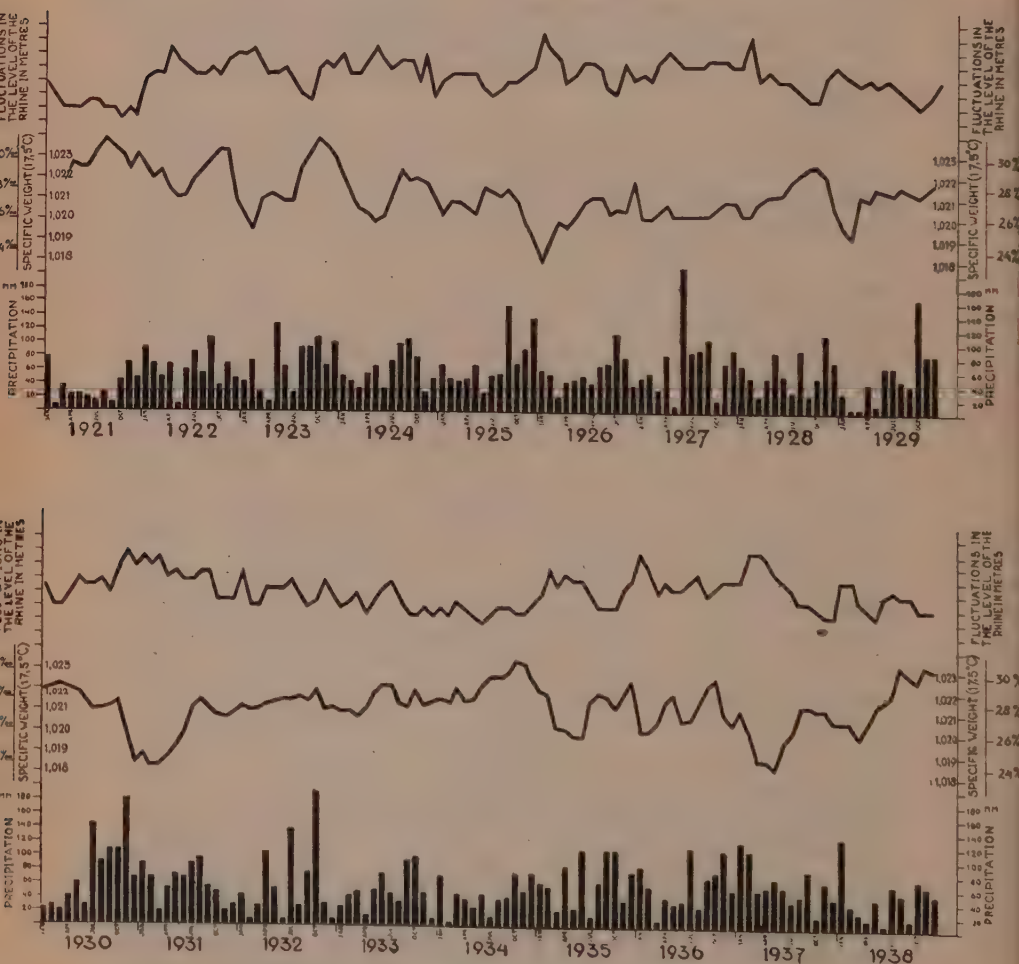
Fig. 3.   The salinity in the Oosterschelde.
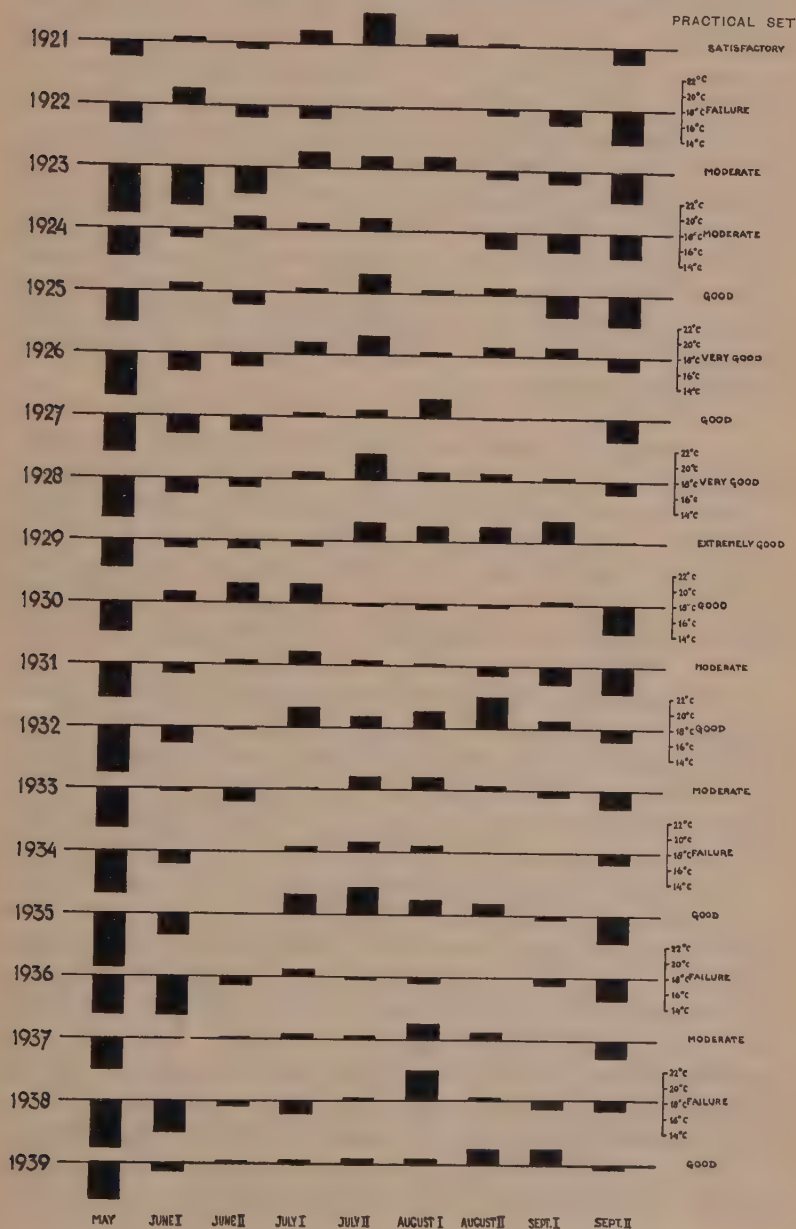
Fig. 4. Watertemperatures during the summermonths.

ascertained twice a day, at high slack water and at low slack
water, the temperature and the salinity of the surface-water in
the basin of the Oosterschelde. These temperature-readings are
exact to half degrees. The salinity is measured by means of an
areometer. I used these data to compute monthly averages of
temperature and salinity for the years since 1921 (table 1, 2
fig. 3). Temperatures taken on or near the tidal lands at low
water may show marked deviations from the temperature in the
bulk of the water, especially during cold or very warm weather.
So I sorted out the temperatures taken on or near the tidal
lands during low slack water. As in this study the water-tempera-
tures during the season of reproduction are of special interest,
I have represented the data for the summermonths (June, July,
August and September) in a more detailed form by computing
half-monthly averages.

    In a special graph (fig. 4) to elucidate this matter I have
tried to bring out when, how long and how much the water-
temperatures rose above the 18 degrees level during these years.
As I shall have occasion to show later on there exists a certain
connection between temperature conditions and the rate of develop-
ment of the oyster larvae. In the tables it may be seen that these
halfmonthly temperature averages have never exceeded 22° C
so far. Practically every summer the water temperature exceeds
the 18° C level for a reasonable period of time.

    Apart from these data, temperatures were taken daily at low
slack water at Kattendijke as well as on the Yersche Bank,
(exact to tenths of degrees) during the summer seasons of the
years 1936-1939. These data are to be found in the diagrams
on the oyster larvae (fig. 7, 8, 9, 10). Generally speaking, low-
water temperatures will show extreme values. The deviation from
the average daily water temperature is not very great, however,
especially not at Kattendijke. On the Yersche Bank, rather close
to the tidal lands, the water-temperature at low slack water
may show values of 1° or sometimes even 2° above or below the
daily average, especially during extremely warm or extremely
cold weather. During the summerseasons of 1938 and 1939
thermograph records of the bottom-water were taken on the
Yersche Bank. These data may also be found in the diagrams
on the larvae (fig. 7, 8, 9, 10). I used a timepiece thermograph
(by Fuess), contained in an iron box, which was placed at the
bottom. The differences between the low-water temperatures

(also taken near the bottom) and the daily averages may be studied from the diagrams on the larvae. Generally speaking, we may state that during summer the low-water temperatures at the Yersche Bank anticipate the average daily water temperatures, with a difference of about one degree. So we see for instance that in the beginning of August 1938 low-water temperatures reached high values sooner than the daily averages and conversely that in the second half of August 1938 low-water temperatures were lower than the daily averages during a period of falling temperature. All these thermograph records have not been reproduced here. They generally show a very regular course with slight peaks during low water. These peaks practically never exceed 1° C. The thorough water mixing by the tides make it impossible for differences between water-temperatures at the surface and near the bottom to subsist throughout a tidal cycle.

As regards the salinity I assume that the areometer-readings effected twice a day since 1921 suffice to form an adequate idea about the salinity in the Oosterschelde. The lowest monthly value of the specific weight recorded was 1,0179 (at 17,5° C) during January 1926. The highest specific weight recorded was 1,0239 during August 1921 and October 1923. On an average the specific weight in the Oosterschelde is 1,0212 (at 17,5°C), which corresponds with a salinity of 27,75 $^0/_{00}$.

Several factors have their influence on the salinity. In the first place we think of precipitation and evaporation as predominating factors. I mentioned before that no volume of fresh water of any importance is discharged into the basin of the Oosterschelde, so only rainwater that has fallen directly into the basin itself has to be taken into account. The salinity of the adjacent waters should not be overlooked, however.

The salinity of the coastal surface water of the North-Sea shows more or less regular seasonal fluctuations. VAN RIEL (1929) states that the 34 $^0/_{00}$ isohaline is to be found at an average distance of 22 km off the shore of Hook of Holland during winter, 44 km during spring, 42 km during summer and 27 km during autumn. The discharge of the great rivers (the Rhine and the Meuse, of which the former is the most important) and the supply of Atlantic water exercise their influence on the salinity of the coastal surface water of the North-sea.

In order to investigate which factor has the greatest influence

TABLE I

AVERAGE WATER-TEMPERATURES IN THE OOSTERSCHELDE SINCE 1921

Computed from temperature-readings twice a day, at high water and

Year	January	February	March	April	May	June I	June II	July I
1921				10,0	15,9	18,5	17,2	19,5
1922	2,0	1,5	5,8	6,6	15,5	20,1	16,3	16,3
1923	3,4	4,9	6,0	10,0	11,9	12,9	14,5	20,0
1924	0,5	1,7	3,3	6,3	14,4	16,8	19,3	18,8
1925	4,6	5,0	4,9	8,8	14,2	18,5	16,7	18,4
1926	3,2	5,0	6,7	9,2	12,4	15,8	16,7	19,4
1927	3,9	2,9	6,5	9,6	13,3	15,6	16,1	18,3
1928	3,2	5,1	6,0	9,9	12,7	15,9	16,8	18,8
1929	0,5	0,2	3,4	7,1	14,6	17,0	17,0	17,3
1930	5,3	3,3	5,1	9,0	14,3	19,2	20,3	20,4
1931	4,0	2,6	4,0	8,6	13,5	16,8	18,3	19,2
1932	4,2	2,1	2,9	7,5	11,8	15,8	17,6	20,3
1933	3,4	1,9	4,7	9,4	13,0	17,7	16,3	18,1
1934	1,3	2,0	4,4	9,2	12,3	16,3	17,8	18,6
1935	3,3	2,7	4,2	7,6	10,9	15,2	17,8	20,4
1936	3,3	1,4	3,6	7,6	12,9	12,9	16,9	18,7
1937	1,8	3,2	4,2	7,6	14,0	18,1	18,2	18,6
1938	3,4	3,5	6,3	8,1	12,1	13,9	17,4	16,5
1939	2,9	3,8	5,6	9,3	13,6	17,1	18,2	18,5
Total monthly averages	3,0	2,9	4,9	8,5	13,3	16,5	17,3	18,7

at low water. Observations at low water on or near the tidal lands excluded.
Observations exact to half degrees. Surface-water used.

Half-monthly averages have been computed for each summer-season.

No observations on account of ice-drift: Dec. 17–23 1927, Febr. 8–March 11 1929, Jan. 23-31 and Dec. 4–21 1933, Jan. 5-16 1934, Dec. 17–28 1938. The real averages for these months may be somewhat lower.

y II	August I	August II	Sept. I	Sept. II	Oct.	Nov.	Dec.
2,0	19,4	18,5	18,1	16,0	14,8	4,4	3,2
7,5	18,0	17,1	16,1	13,6	9,7	5,3	4,8
9,5	19,6	17,0	16,5	13,9	11,9	4,9	2,4
9,5	18,7	15,9	15,7	14,8	12,6	7,5	5,1
0,3	18,5	18,7	15,2	14,2	12,3	5,8	1,1
0,3	18,4	19,1	19,1	16,7	12,6	6,9	5,2
3,9	20,5	17,8	18,1	15,2	12,6	9,6	3,3
1,1	19,0	18,8	18,5	16,4	12,3	9,3	5,1
0,5	19,9	20,1	20,7	18,0	13,0	7,6	5,1
7,5	17,4	17,6	18,3	14,3	11,9	8,6	4,6
3,4	18,2	16,9	15,8	14,6	12,8	8,2	5,7
0,4	20,2	22,1	19,0	16,5	11,8	7,5	3,9
7	19,7	18,6	17,3	15,8	12,5	6,2	0,1
1	18,7	17,9	18,0	16,6	12,0	6,4	6,1
6	19,8	19,4	17,4	14,3	10,6	7,0	1,9
6	17,1	18,0	17,0	15,2	9,2	5,3	2,0
5	20,0	19,0	18,0	15,8	12,7	7,3	3,1
5	22,1	18,6	17,2	17,0	12,7	9,8	4,5
7	18,7	19,8	20,0	17,6	11,4	7,9	2,1
5	19,1	18,5	17,7	15,6	12,1	7,1	3,6

TABLE II

MONTHLY AVERAGES OF THE SPECIFIC WEIGHT OF THE WATER IN THE OOSTERSCHELDE SINCE 1921

Specific weight at 17,5° C.

Year	January	February	March	April	May	June	July
1921			1,0218	1,0227	1,0226	1,0226	1,0232
1922	1,0227	1,0220	1,0223	1,0214	1,0211	1,0212	1,0219
1923	1,0202	1,0194	1,0209	1,0212	1,0211	1,0209	1,0209
1924	1,0220	1,0211	1,0206	1,0204	1,0198	1.0202	1,0213
1925	1,0202	1,0208	1,0209	1,0206	1,0203	1,0215	1,0214
1926	1,0179	1,0188	1,0199	1,0197	1,0201	1,0208	1,0211
1927	1,0201	1,0201	1,0204	1,0206	1,0202	1,0203	1,0203
1928	0,1202	1,0202	1,0209	1,0211	1,0211	1,0212	1,0219
1929	1,0195	1,0191	1,0212	1,0210	1,0215	1,0214	1,0213
1930	1,0218	1,0220	1,0222	1,0221	1,0218	1,0214	1,0209
1931	1,0188	1,0182	1,0183	1,0187	1,0193	1,0200	1,0212
1932	1,0212	1,0211	1,0211	1,0215	1,0216	1,0217	1,0217
1933	1,0211	1,0211	1,0208	1,0212	1,0218	1,0223	1,0223
1934	1,0217	1,0216	1,0222	1,0216	1,0217	1,0223	1,0227
1935	1,0220	1,0218	1,0202	1,0201	1,0198	1,0198	1,0216
1936	1,0220	1,0202	1,0206	1,0215	1,0218	1,0204	1,0206
1937	1,0211	1,0201	1,0187	1,0187	1,0184	1,0197	1,0202
1938	1,0207	1,0207	1,0199	1,0206	1,0215	1,0216	1,0220
1939	1,0230	1,0221	1,0212	1,0202	1,0208	1,0210	1,020'
Total Monthly Average	1,0209	1,0206	1,0207	1,0208	1,0209	1,0211	1,021.
Salinity °/oo	27,4	27,0	27,1	27,2	27,4	27,6	28,0

Computed from daily areometer-readings. (Surface-water used).

No observations on account of ice-drift:

Dec. 17–23 1927, Febr. 8–March 11 1929, Jan. 23–31 and Dec. 4–21 1933, Jan. 5–16 1934, Dec. 17–28 1938.

ugust	September	October	November	December	Year-average	Rain-fall (total)
0239	1,0234	1,0231	1,0223	1,0231	1,0229	405 mm
0225	1,0230	1,0223	1,0223	1,0209	1,0220	757 mm
0225	1,0232	1,0239	1,0236	1,0228	1,0217	860 mm
0223	1,0216	1,0217	1,0215	1,0209	1,0211	736 mm
0212	1,0215	1,0210	1,0198	1,0190	1,0207	874 mm
0212	1,0202	1,0205	1,0204	1,0218	1,0202	696 mm
0203	1,0202	1,0206	1,0208	1,0208	1,0204	915 mm
0223	1,0225	1,0226	1,0222	1,0208	1,0214	736 mm
0216	1,0214	1,0212	1,0214	1,0217	1,0210	693 mm
0210	1,0212	1,0215	1,0202	1,0183	1,0212	906 mm
0215	1,0211	1,0207	1,0206	1,0209	1,0199	702 mm
0218	1,0215	1,0220	1,0210	1,0212	1,0214	735 mm
0213	1,0212	1,0220	1,0214	1,0216	1,0215	601 mm
0227	1,0228	1,0235	1,0233	1,0227	1,0224	592 mm
0219	1,0217	1,0212	1,0218	1,0224.	1,0212	802 mm
0215	1,0223	1,0226	1,0209	1,0205	1,0212	733 mm
0214	1,0214	1,0212	1,0211	1,0207	1,0202	790 mm
0233	1,0229	1,0227	1,0233	1,0232	1,0219	586 mm
0209	1,0218	1,0215	1,0196	1,0186	1,0210	
0218	1,0218	1,0218	1,0214	1,0213	**1,0212**	
28,5	28,5	28,5	28,0	27,9	**27,75**	

on the salinity in the basin of the Oosterschelde I have composed
a diagram (fig. 3). The average monthly values of the salinity
of the surface water in the basin of the Oosterschelde and the
corresponding values of the specific weight at 17,5° C are recorded
in the intermediate graph of the diagram. (N.B. No observations
on account of ice-drift: Dec. 17–23 1927, Febr. 8–March 11
1929, Jan. 23–31 and Dec. 4–21 1933, Jan. 5–16 1934, Dec. 17–28
1938). The total monthly precipitation in mm at Bergen op
Zoom is recorded below. These data were placed at my disposal
by the Koninklijk Nederlandsch Meteorologisch In-
stituut at De Bilt. (N.B. The observations during Dec. 1925,
Febr. 1929, March 1929, April 1933 and October 1938 were
carried out at Poortvliet, 12 km N.W. of Bergen op Zoom, and
the observations during December 1938 at Krabbendijke, 14 km
S.W. of Bergen op Zoom). The graph at the top of this diagram
shows the fluctuations in metres of the waterlevel of the river
Rhine. The Rhine is the most important fresh water dis-
charging river in Holland. I assume that the height of the water-
level recorded for instance some 100 km from the shore, may
be regarded as an approximate standard for the volume of water
discharged. The average monthly values of the waterlevels can
be found in the "Jaarboeken der Waterhoogten" by Rijkswater-
staat. So the topmost graph may be considered to indicate fluct-
uations in the discharge of the river Rhine. I cannot demonstrate
variations in the intensity of the evaporation in this diagram, as
I have no data about them at my disposal.

Studying this diagram, we shall find that regular seasonal
fluctuations in salinity do not occur here. The local precipi-
tation does not show a marked relation with the variations in
salinity. Often sharp fluctuations in the rainfall fail to evoke a
reaction of the salinity, sometimes the salinity even increases
during a very wet month. Fluctuations in the discharge of the
Rhine, on the other hand, have a well-marked influence on the
salinity in the basin of the Oosterschelde. Generally speaking,
we may state that the graph on the waterlevel of the Rhine may
be considered to be approximately the reflected image of the
graph on the salinity.

So I want to conclude that the discharge of the Rhine has a
marked influence on the salinity in the basin of the Oosterschelde
and that the local precipitation is of much less importance in
this respect.

IV. AIMS OF INVESTIGATION

In connection with the enforced return to the old approved system of tile-collectors in the Zeeland waters and with the very difficult position of the local oyster industry in the years following 1930 support was rendered to the oyster farmers by elaborating a system of forecasting the periods of spatfall in order to increase the chance of obtaining a sufficient spatfall on the tiles (HAVINGA, 1938, 1939).

The primary purpose of these investigations was to make an analysis of the maxima and minima of swarming in the course of the season of reproduction and of the pelagic life and the setting habits of the oyster larvae with reference to environmental conditions and the demands on the nature of the collector-material.

Especially our knowledge of the free-swimming stage of the larvae of *Ostrea edulis* showed many gaps. Though many investigations have been carried out on the biology of the larvae of other kinds of oysters during the pelagic stage, especially in America, it should not be assumed that the larvae of *Ostrea edulis* will act in quite the same way as those of the foreign species. It is impossible to understand the correlation between swarming and setting without a thorough knowledge of the interjacent stage, in casu the pelagic stage. So special attention was paid to the biology of the free-swimming larvae. Because but few reliable scientific data concerning the demands that mature larvae of *Ostrea edulis* make upon the nature of the cultch material are available, this subject was also inquired into. Although larval life and the setting process were studied simultaneously, I shall discuss these different subjects separately. The correlation between swarming and setting, the most important point in connection with the aims of these investigations, can only be discussed after an elaborate review of the methods and results of the inquiries into these separate subjects.

Spawning and swarming determine when and where young larvae can be expected, while still other factors determine when and to what degree spawning will occur. Therefore a discussion of the processes preceding spawning should not be lacking here for a full understanding of the entire process.

V. METHODS – INVESTIGATION ON THE PELAGIC STAGE OF THE LARVAE

Recognition of the larvae

A conditio sine qua non in the study of planktonic organisms like oysterlarvae, is the possibility of an infallible recognition of such larvae among the herds of other planktonic organisms, which will be found to include many larval stages of other kinds of Lamellibranchs. Notwithstanding the fact that the larvae of Bivalvia form one of the few groups of planktonic organisms, about which no extensive systematic literature[1]) exists, the recognition of the pelagic larvae of *Ostrea edulis* does not present any difficulties. Morphology and colour of the larvae of *Ostrea edulis* are very characteristic. Moreover the larvae of this incubatory species of oyster do not make their appearance in the plankton till they have attained a clearly recognizable stage, as the first part of their larval live is spent in the maternal mantle chamber.

Though investigators in the nineteenth century (e.g. HORST 1884) did not succeed in finding oysterlarvae in the plankton, no recent complaints about difficulties in identifying the pelagic larvae of *Ostrea edulis* are known to me. Without doubt living larvae, which show their natural colours, are easier to recognise, but well-trained eyes can also identify colourless preserved larvae. Though at first the simultaneous appearance of larvae of *Ostrea edulis* and of *Gryphaea angulata*, the Portuguese oyster, in the basin of Arcachon, presented some difficulties, BORDE (1930), the local oyster investigator, soon detected the difference between the larvae of those two kinds of oysters. The larvae of *Gryphaea angulata* closely resemble those of *Ostrea virginica*. With the aid of the figures of the larvae of *Ostrea virginica* in WELLS' paper (1927) BORDE was able to bear out his statement.

The identification of the pelagic larvae of some other kinds of oysters does not seem to present serious difficulties. After the paper of STAFFORD (1912) on the recognition of bivalve larvae, many authors (e.g. NELSON, 1921, WELLS, 1927) have discussed the features by which the larvae of *Ostrea virginica* can be identified. SENÔ (1929) and SEKI (1930) tell us how the larvae of *Ostrea denselamellosa* are to be recognized.

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[1]) Recent contributions to the systematics of the larvae of Lamellibranchs are e.g. the papers by KÄNDLER (1927), LEBOUR (1938) and WERNER (1939).

Quantitative plankton-samples:

It is easy to understand that only a comparison of comparable plankton- samples can give reliable information about the amount of oysterlarvae in the water, about the growth of those larvae, their movements and the amount of mature larvae.

Ostrea edulis.

When we consider the methods used in studying the larvae of the common European oyster, *Ostrea edulis*, our attention is in the first place directed to France, where planktonic oyster-larvae have been investigated since 1922.

In France samples are invariably collected by means of a plankton-net. After preliminary studies in the first years (LEENHARDT 1922, 1924) a uniform plankton-net and a uniform sampling method were introduced at several stations on the French coast. A description of how these investigations have developed in the French oyster localities is given by LAMBERT (1935) and by LADOUCE (1938 c).

In France a standard plankton-net, of a standard model, of standard measurements and made of a standard bolting silk (since 1924 no. 130, in the first years no. 140), is used. The construction of this net is rather primitive, however, and lacks, for instance, a tap to draw off the plankton. To procure comparable samples it is necessary to try to eliminate as many of the inconstant factors as possible. To eliminate the influence of the tidal cycle the samples in France are preferably taken at the same stage of the tide (at half flow). To filter off with the net approximately the same volume of water the French investigators tow the net against the stream for a certain time (five minutes) at approximately the same speed. The volume of filtered water is not known. The towing is carried out along the surface of the water ("à une vitesse telle qu'il restait au surface sans sortir de l'eau et faisant un sillage"). As the surface-layers of the water show the most extreme variations in salinity (rain-showers) and temperature, a possible migration of the larvae in a vertical direction will show a maximum amplitude in those surface-layers. In their endeavours to eliminate such migrations some investigators in France have sampled with a double net in recent years, one along the surface and one somewhat deeper (VOISIN, 1932). This problem will be discussed in the chapter on vertical migrations. To avoid floating eel-

grass at Arcachon, the net was towed about 10 to 15 cm below the surface in 1937 and in 1938 (LADOUCE, 1938 a, 1938 b).

Reliable observation of the emission of larvae into the plankton and of their subsequent growth is only possible when samples are collected very frequently. Daily sampling is very desirable. Although the French investigators aim at daily sampling, they are handicapped by practical and technical difficulties. The consequence is that in some localities sampling takes place only once or twice a week, which results in a less reliable comparison of the data obtained.

The plankton samples collected in this way are diluted with water to a certain volume and in known quantities of this thoroughly shaken mass the oyster larvae are counted with a microscope. With the aid of the data obtained in this way the total amount of oysterlarvae in the original plankton sample is computed. BOURY (1928) tells us that countings which seem too extreme are eliminated! Such a proceeding is open to criticism.

In France the oyster larvae are not measured regularly. In recent years however, some French investigators distinguish and count the so-called larvae in the second stage. This division is equivalent to the classification "straight-hinge-" and "umbo-larvae" given by other writers. I wonder with KÄNDLER (1928) why the French investigators omit measuring the oyster larvae in the plankton-samples, which procedure may give very important information concerning growth, age and loss of those larvae. Where the larvae of *Ostrea edulis* and *Gryphaea angulata* occur simultaneously, as in the bassin d'Arcachon, the totals of both kinds of larvae in the samples are often added up. Diagrams composed of such data (BORDE) cannot possibly give clear information.

The results of the French investigations are to be found in the papers of LEENHARDT (1922, 1924), BOURY (1928, 1929 a, 1930), RAPHENNE (1930, 1931), VOISIN (1931, 1932), TACLET (1932, 1935), HERMAN (1935, 1936, 1937, 1938 a, 1938 b) all of them on the waters in the Morbihan. Further CHAUX-THÉVENIN (1931–1938) (*Gryphaea angulata*) at Marennes and at Arcachon BORDE (1929–1937) and LADOUCE (1938 a, 1938 b).

Other quantitative studies on the planktonic larvae of *Ostrea edulis* in open waters (I leave out the frequent checking of the amount of larvae in enclosed ponds or pits that went with the

attempts in England, Germany and Denmark to effect "arti-
ficial" propagation) can be found in the papers by KÄNDLER
(1928) and HAGMEIER and SCHUBERT (1930) on investigations
in the German oystergrounds, by SPÄRCK (1925) on the Lim-
fjord-plankton and by GAARDER (1932) on studies in the Nor-
wegian oyster pools.

SPÄRCK (1925) found but very few larvae in the Limfjord
waters and in fact only once detected a mature larva in the
samples obtained with his plankton-net.

The German investigators use a plankton-net of a special
construction. The large quantities of detritus in the Wattenmeer
often cause a rapid obstruction of the meshes of the bolting silk
and make it impossible to obtain quite reliable quantitative
plankton-samples. KÄNDLER (1928) likewise judges it impossible
to estimate approximately the volume of water filtered during
the five minutes' towing. The German plankton-investigations
were carried out in the summers of 1926, 1927 and 1928, after
the importation of Dutch seed-oysters in the German oyster-
grounds. The larvae in the plankton samples were counted and
measured without preliminary dilution. The sampling did not
take place very frequently, but only a few times during the season
of reproduction. Though the volumes of filtered water in the
German samples cannot be compared with those in the French
samples and in either case cannot even be estimated approxi-
mately, it will be clear that during the years of investigation in the
Wattenmeer far fewer larvae per unit of water could be found
there than at the French sampling-stations. In France thousands
of larvae occur in one sample and in the Wattenmeer seldom
more than a hunderd, both samples being obtained by five min-
utes' towing; in the Wattenmeer even with a larger net than in
the French waters.

GAARDER (1932) studied quantitative plankton samples from
two Norwegian oyster pools (Espevik-poll and Saelø-poll). The
exceptional hydrographic conditions in those pools (no tidal
cycle and a marked stratification of the water) present other
possibilities. The oyster larvae are only present in the deeper
salt layers. Sampling was carried out by pumping up ten litres
of water from the deeper layers and filtering them with a plank-
ton-net. Samples from different depths were taken five times
during the season of reproduction. All the larvae were counted
and measured.

Before describing the methods of plankton-sampling in Holland I will briefly discuss the methods used in America. Very curious data have been obtained by some American investigators.

On the Atlantic coast samples of the larvae of *Ostrea virginica* are collected quantitatively. The first quantitative sampling was carried out by filtering a pail of water through a plankton-net (NELSON, 1917). Though some investigators obtain quantitative samples by towing a plankton-net (GALTSOFF, 1930), in most cases a known quantity of water (from 50 litres to 200 gallons) is pumped up from a known depth and filtered through a plankton-net (CHURCHILL, 1921, NELSON, 1921, PRYTHERCH, 1929, PERKINS, 1931). In the southern part of the range of *Ostrea virginica* HOPKINS (1931) carried out investigations. A quantitative method of measuring the abundance of oyster larvae in plankton-collections was not employed, for while such measuring might be feasible during a few weeks, it would take too much time to continue it over a period of several months. A crude method (plankton-net-tow) was devised, which gave results of relative value.

In some localities the plankton-samples always contain numerous larvae during the season of reproduction. Some investigators sieve off the oysterlarvae with monel-metal screens before counting them (CHURCHILL, 1921, PRYTHERCH, 1929); sometimes they are counted and measured (CHURCHILL, 1921); often the larvae are only counted, the occurrence of mature larvae being recorded (NELSON). It is very curious that, though often thousands of larvae may occur in 100 litres of water in some localities (Barnegat Bay, Great South Bay), other investigators fail to detect any larvae in many samples collected in other places (Wareham River, Onset Bay, Milford Harbor), only a few larvae being found in the other samples (GALTSOFF, PRYTHERCH, MC.MILLAN, 1930, PRYTHERCH, 1929). This is not a question of rich and poor breeding localities, for even in those places where few larvae are found in the plankton-samples an extraordinarily profuse setting may occur. The cause of this remarkable difference will be discussed in the chapter on vertical migration.

No quantitative studies on the amount of the larvae of *Ostrea lurida* have been published so far.

The methods of plankton-sampling that have been in use in the Oosterschelde (Holland) since 1935, have been worked out by HAVINGA (1938, 1939). A quantitative sample is obtained by pumping up 100 litres of water from a known depth and by filtering them at once through a specially constructed plankton-net (bolting silk Nr. 130). The plankton is drawn off from the net by means of a tap. The sampling is carried out daily at low slack water to eliminate as far as possible the influence of the tides. A total elimination of the influence of the tidal movements would be impossible, as one cannot get round the difference between spring-tides and neap-tides and the influence of strong winds on the currents.

A discussion of the possible influence of the depth of sampling can be found in the section on vertical migrations.

The sampling is carried out at two stations of different character, the first being the centre of larvae-production, while the second, an important spatfall-centre, receives all its larvae through the tidal watermovements. During the most important part of the reproduction-season daily sampling is carried out. In the beginning and during the last part of the season the sampling takes place every second day. The daily samples are studied alive, as living oysterlarvae are more easily recognisable than preserved ones. It has appeared to me that no mortality or loss of oysterlarvae occur in such living samples, if we let for instance 24 hours elapse between sampling and microscopical examination. With the aid of a counting glass and a counting table the entire sample is searched for oyster larvae, which are all of them counted and measured (the greatest length of the shell parallel to the hinge), while the presence of larvae bearing a pigment-spot (mature larvae) is recorded.

Movements of the larvae under the microscope are prevented by means of a coverglass.

Apart from the daily sampling many groups of other quantitative plankton-samples have been collected during the season of reproduction in the years 1937 and 1938 for the sake of inquiries into movements of oyster larvae in horizontal and vertical directions.

As the study of the daily samples requires a good deal of time, it was impossible to count and measure the oyster larvae in these special samples alive. Therefore I preserved these samples of larvae in alcohol; corrosion of the shell-edges of the larvae, which often takes place in formaline-solution, does not occur in

alcohol. Alcohol causes precipitation of the calciumsulphate
in seawater as a thick whitish mass. Before microscopical investi-
gation is proceeded to, dilution with water will dissolve this
troublesome gypsumprecipitate.

In my opinion the pump-method gives better quantitative
results than plankton-net-towing: the depth of sampling and
the volume of filtered water are absolutely under our control
in this way.

I am aware that objections can be raised against the use of
a pump and suctionhose. Excellent apparatus for the collection
of indisputably reliable quantitative watersamples at a known
depth exists, but these methods are only practicable in the study
of minute and abundant plankton-organisms. Samples of 100
litres cannot easily be collected with this apparatus.

As regards the pump-method we can find a discussion of the
use of this kind of gear in the paper by Gibbons and Fraser
(1937); they compared the results obtained with the pump-
method with those of several kinds of plankton-nets.

They prefer a pump for the study of non-motile and minute
forms, but nets are considered better for the collecting of samples
of fish-larvae and other motile forms.

Limnologists know that many little Crustacea tend to swim
against the direction of the current stirred up around the mouth
of the suction hose. The speed with which the hose sucks in the
water will vary with the power of the pump. We may imagine
a number of concentric spherical zones around the mouth of
the suction-hose, in which the speed of the flowing water will
vary. The nearer to the hose-mouth the greater the speed. It
will be impossible for little plankton organisms in the inner
zones to counteract this current by their own motive force. In
the outer zones the speed of the flowing water will be so tardy
that those animals which tend to swim against the stream are
able to escape the suction-hose, which will make the sampling
unreliable.

These arguments only apply to stagnant waters. When the
mouth of the suction-hose moves in respect to the water with
a s u f f i c i e n t speed, those zones do not endure long enough
in one place to make such an escape possible and the sampling
will be more reliable. So pumps can be used in streaming water
and also from a moving boat in stagnant water.

Though hitherto it was not known how oysterlarvae will

behave in this respect, there is a possibility that their behaviour will be similar to that of the said Crustacea, of which the consequence would be unreliable sampling in stagnant water. As the tidal movements of the waters in the Oosterschelde cause strong currents (see hydrographical conditions), these objections against the pump method cannot be raised here. Later on I shall prove that even during slack water pump-sampling of oyster larvae yields trustworthy quantitative results (see horizontal movements). In how far a reliable comparison of the daily plankton-samples is justified will also be discussed in that section.

To prevent the whirling of bottom material in case of a too close proximity of the hose-mouth to the bottom, which would render microscopical examination of such plankton-samples practically impossible, a leaden plate, about a square foot in size, is attached horizontally a few inches under the mouth of the suction hose. The mouth is protected against the invasion of undesirable material, such as seaweed and jelly-fishes, by means of a piece of copperwire with fairly wide meshes.

VI. SPAWNING

Spawning act

Female spawning involves the discharge of the eggs from the gonad into the suprabranchial (= cloacal) chamber.

In non-incubatory species of oysters an extrusion of the eggs from the inhalent chamber into the free seawater immediately follows on spawning, while in the incubatory species of oysters this extrusion is postponed till the larvae have reached a certain stage of development. It is a very remarkable fact that those non-incubatory species extrude the eggs via the inhalent chamber instead of following the seemingly much easier way direct from the exhalent chamber (= suprabranchial chamber) into the outer world, i.e. the way followed by the exhalent water, the sperm and the waste products of rectum and kidneys.

This points to a close relationship of those two types of oysters. The only difference between incubatory and non-incubatory oysters as regards the way in which the offspring leaves the mother-oyster lies in the time required for the entire process.

The second stage of this event, the discharge of the eggs from the inhalent chamber into the seawater, has been observed more than once in *Ostrea virginica*. NELSON (1921) gives a good des-

cription of his observations; he was, however, not the first to witness the spawning of this kind of oyster. Each female, in spawning, relaxes the adductor-muscle until the shells gape to their widest extent and then by a quick contraction of the adductor muscle expels the water within the shells and with it a great quantity of eggs. With each expulsion, at intervals of about 30 seconds, the eggs are driven out in a fan-shaped cloud.

In another paper by NELSON (1922) figures of spawning oysters will be found, together with the reproduction of a chimograph-tracing of the shell movements of a spawning female oyster. The shells of these oysters were attached to a recording apparatus used for observing the water current caused by the gills and by good fortune the rhythmic contractions by which the eggs are shot out could be recorded. Similar chimograph records of the spawning actions of *Ostrea virginica* and *Ostrea gigas* will be found in the papers by GALTSOFF (1930 a, 1932). While the eggs are discharged by violent shell movements, the males show no shell movement whatever, but remain quiet while spawning, with the shells gaping, while on the dorsal or upper side about midway between the hinge and the tips of the shells (exhalent chamber!) a steady stream of milt issues from between the shells (NELSON, 1921, figures in NELSON, 1922). GALTSOFF (1930 a, 1932) describes a similar action, the washing out of the sperm through the cloaca with the water pumped by the gills, both in *Ostrea virginica* and *Ostrea gigas*.

The first stage of the spawning process, however, the action by which the eggs pass from the gonad to the suprabranchial chamber and thence from the suprabranchial chamber to the branchial chamber, has never been observed directly. So far the only plausible explanation is the passage of the eggs from the suprabranchial chamber through the water-tubes and gill-slits to the branchial chamber.

In accordance with this explanation is the discovery of ELSEY (1935) that the gillslits (or ostia) of *Ostrea lurida* and *Ostrea gigas* have a diameter proportional to that of their eggs. It is difficult to understand in what way the eggs are forced through the gill-apertures. STAFFORD (1915) suggests pressure, but perhaps the opinion of GALTSOFF that suction caused by opening the valves during spawning draws the eggs through the ostia is more satisfactory. Just as the first stage of the spawning process in

Ostrea virginica has escaped observation so far, no investigator has as yet observed the extrusion of the eggs into the branchial chamber in incubatory species, such as *Ostrea edulis* and *Ostrea lurida*. Attention has been called to this gap in our observations by Hopkins (1937) as far *Ostrea lurida* is concerned and by Hagmeier (1931) and Orton (1937 a) for *Ostrea edulis*. The latter again suggests pressure as effecting the passage through the gill-slits, by "contraction of the posterior mantle and gill-muscles, which obliterate the cavity of the exhalent chamber and forces the eggs into the inhalent".

The delayed second stage of the spawning-act in the incubatory species will be described in the section on "swarming".

Periodicity of spawning

Oysters show a more or less regular periodicity in spawning. The spawning season may vary in length to a high degree, according to the local climatological conditions. The length of the season of reproduction has been discussed already in the section on the range of the oysters. As regards the two types of oysters different methods of observation of this periodicity have to be applied.

Spawning in non-incubatory species can be observed by following the quantity of oysterlarvae in the plankton, which often shows marked increases. In some of the culture-regions of these oysters (e.g. *Ostrea virginica*) nearly all the spawning of the season tends to be concentrated in one or two days, while in other regions the spawning of the same kind of oyster is not limited to a few days, but is distributed over many spawning-days of different importance. As far as incubatory kinds of oysters are concerned, it is clear that a certain space of time separates spawning from the increase of larvae in the plankton; the length of this time is dependent on the duration of the incubation. The only reliable method of studying the periodicity of spawning in incubatory oysters directly is a regular examination of the percentages of adults carrying young in comparable samples of oysters. The spawning periodicity in *Ostrea edulis* has been investigated especially by Orton.

This periodicity is brought about by the stimulating activity of two factors of a different nature. Temperature is one of these factors, while a stimulation of a chemical nature may be exercised by the sex-products of other oyster individuals, at least in

some species of oysters. The mature egg or sperm must practically be ready for extrusion for any kind of stimulation of spawning to be possible.

There is often much difference in the stage of development of the gonads in different individuals of *Ostrea edulis*. This complicated matter can only be elucidated by a preliminary treatise on the phenomenon of sex-change. So I shall have to deal with the internal and external influences on the periodicity of spawning separately.

VII. INTERNAL FACTORS GOVERNING THE PERIODICITY OF SPAWNING

Ostrea edulis.

In the days of DAVAINE (1853) and LACAZE-DUTHIERS (1855) the first discoveries about the hermaphrodetic character of *Ostrea edulis* were made. MOEBIUS (1871) records the development of sperm after the shedding of the eggs.

The beautiful microscopical studies by HOEK (1884) provided us with further information. He isolated a number of oysters carrying larvae and picked these oysters up again after one to four weeks. Microscopical investigation showed development of sperm in all of them. The eggs in one female oyster always show the same stage of development, so most probably all the eggs are extruded simultaneously. The stages in the development of the sperm, on the other hand, will differ. The presence of ripe sperm usually accompanies the appearance of earlier stages of sperm-development. So HOEK declared that the shedding of sperm will take place during a much longer period than the extrusion of the eggs. HOEK rejects the possibility of self-fertilization.

Though this study meant an advance in our knowledge, HOEK was not aware of the entire sex-cycle and the laws governing sex in *Ostrea edulis* were not to be discovered till forty years later.

Recent researches by ORTON and SPÄRCK cleared up a great deal of the confusion. Both ORTON and SPÄRCK realized that no observation of the actual stage of the gonad could ever give clear information about a succession of sex-phases, no matter the amount of oysters examined. Repeated examination of the gonad of the same oyster individuals, for instance in the course of one year, would be necessary. They discovered that the boring of a hole in one of the shells and the extraction of a portion of

the gonad-tissue for microscopical examination did not do the oyster any harm.

While ORTON sorted out a certain amount of oysters showing the same stage of sex and isolated these identified oysters in the sea in cages (1922 a), SPÄRCK isolated single examined individuals in aquaria (1925). The assertion of DAVAINE (1853) that the first sexual phase of the oyster is the male one was confirmed (ORTON, 1921).

The change from female to male was investigated in oysters of ages varying between one and six or seven years. Female functioning oysters of any of these ages appeared to change their sex at the time of spawning from female functioning to maleness (ORTON, 1921, 1922 a, SPÄRCK, 1925). The fact that practically every oyster shows a development of sperm shortly after the shedding of the eggs prevents the production of a second batch of larvae during the period of femaleness, at any rate as a rule. When some eggs happen to remain in the gonad after the general extrusion, they will be absorbed or eliminated as an excretion-blister (ORTON, 1927 d).

The change from female to male sets in very quickly after the shedding of the eggs and at the time of swarming practically every oyster contains mature sperm-morulae (ORTON, 1927 c, 1927 d). The way in which this change takes place resembles an automatic reaction. The culminating point in the production of sperm is reached about a month after this sex-change (ORTON, 1927 d). When the extrusion of the eggs takes place towards the end of the season of reproduction and the temperature is falling rapidly, the subsequent development of the sperm fails to take place and gonad-action comes to a standstill till the next spring sets in (SPÄRCK, 1925). In the paper by ORTON and AMIRTHALINGAM (1931) some figures on the development of sperm during incubation can be found.

The next point to be investigated was whether these individuals which have changed from female functioning to maleness remain permanently male or change back to femaleness. If all the females change into males, the proportion of males should increase with age, unless a reversion to the female stage occurs. A study of the proportion of the two sexes at various ages (ORTON, 1936) clearly shows that maleness does not increase with age and establishes indirectly that sex-change from male to female must occur. The occurrence of this sex-change was proved with the

aid of the boring-device. ORTON (1933) isolated his male oysters in cages in the sea, while SPÄRCK (1925) observed isolated individuals in aquaria. Though many of ORTON's oysters did not reach the date of examination in the next year alive, the presence of a certain amount of indubitable females among the surviving oysters proved the occurrence of a sex-change from male to female, even under unfavourable conditions. These oysters were left unexamined during a year and we do not know what happened in the course of that year. ORTON states (1933) that he did not expect a rapid reversion and that probably many of these oysters passed the female stage undetected. His experiments were not conducted with a view to detecting a rapid development of the female phase. He presumes that under wholly natural conditions it is highly probable that the percentage of males changing into functioning females within the period of one year is much greater than that found under his experimental conditions.

SPÄRCK (1925) succeeded in obtaining some data on the rate of change from male to female. Though this reversion does not take place as quickly and automatically as the change from female to male, he found that this change may be completed within a few weeks. Thus SPÄRCK observed for example a male oyster (June 6) which contained sperm as late as July 8 and which was found to be female on July 15. Another individual was male on June 20, female on July 4 and carried young on July 25.

As the eggs develop, the oysters remain functioning as males for a considerable time and often sperm-production continues till about ten days before the discharge of the eggs! (SPÄRCK 1925). When we consider that a few days after the shedding of the eggs the gonad again contains sperm-morulae, we may conclude that the purely female phase can be very short (about three weeks).

Knowing this, we shall not be astonished at finding far more males than females in practically every sample of oysters.

As the first sex-phase in an oyster is always the male one, which is reached in its second summer (ORTON (1922 a) once met with a male at the age of 23 weeks), the first change which will take place is the change from male to female. After many contradictory statements in the past concerning the age at which an oyster can reproduce as a female it was proved in recent years that the farther north, the later the first sex-change will occur.

Spärck (1925) states that in the Limfjord no female oysters younger than three or four years can be found and that a first reproduction as a female at the age of four is more common than at three.

Orton (1922 a) tells us how he once found an oyster carrying young in his second summer after the exceptionally fine summer of 1921. After the fine summer of 1935 the occurrence of female oysters in their second summer in the English waters was established (Dodd, Mc.Cloy, Davies, Edmonds and Orton, 1937). In the course of my investigations in the Oosterschelde I noticed more than once an oyster in his second summer carrying larvae. In Holland a fine preceding summer is not a necessary condition for such a rapid female development, but all the same I consider female reproduction in the second summer as exceptional in the Zeeland waters.

In the warmer waters of France female reproduction in the second summer is no exception (Gerbe, 1876, Dupain, 1932). The result of this difference in the age at which the first sex-change takes place is a much higher percentage of male oysters in samples taken in the northern waters, at least if young oysters are not excluded. We do not possess adequate information about the natural frequency of sex-change and about the period normally required for an entire cycle in the various regions of oyster culture. The frequency of sex-change is probably affected by the temperature and possibly by the amount of nourishment. (Spärck, 1925 and Orton, 1936).

In winter the sex-change is checked. Spärck (1925) states that the development of sex-cells is practically interrupted from September 1 till the beginning of April in the Limfjord. The warmer the water, the shorter the interruption will be, which corresponds with the greater length of the season of reproduction in the southern regions.

A fall in temperature in autumn will check the sex-phenomena, though as yet we do not exactly know when. The stage of the gonad at the moment of interruption will be the stage during hibernation. When the temperature rises in spring, sex will revive at a certain temperature. The oyster recommences at the sex-stage at which it left off in autumn. Indeed a certain percentage of the oysters will hibernate as females, the gonads containing eggs, others as males, the gonads containing sperm, while again others do not show any sex-products at all during

that period (Spärck, 1925; Orton, 1927 d). The last category
probably consists mainly of oysters that spawned the last time
as males. Before egg-development begins a certain period of rest
and fattening is often assumed (Orton 1927 c, Orton, 1937 a).
Though the change from male to female is certainly less rapid
than the reversed one, the occurrence and length of such
a period of recuperation has not yet been established. We
know that the change from male to female can take place
fairly quickly (Spärck, 1925), but we know too little as yet
about the influence of temperature and nourishment on this
change.

Spärck made an attempt to study the influence of temperature
on the sex-change in aquaria with heated water. He was ham-
pered in this by a high mortality of his oysters and great difficulties
with feeding, especially during the winter-months.

A very frequent examination of the gonad under conditions
as natural as possible might give further information about the
frequency of sex-change in different localities, so under different
conditions of temperature and nourishment.

Orton (1924) informs us that he observed an oyster which
passed the female stage twice during one season of reproduction.
This oyster spawned again as a female six weeks after it was
last found to be in a male condition. Orton (1926) with the
aid of data on the percentages of oysters carrying young (in
weekly samples) computes that each adult oyster will function
as a female at least once every year. His data (collected in 1925)
show that more than 100% of the adult oysters carried larvae
during that season, so that a certain amount of oysters must
have passed the female phase twice. I believe that this phenome-
non, i.e. the functioning as female for the second time during
one reproduction-season, is not exceptional in the Oosterschelde.
I base this assertion on the fact that production of larvae can
take place in the Oosterschelde for a considerable period every
summer, often showing several optima, as well as on the fact
that the average temperature conditions are more favourable in
the Oosterschelde than in England. So when Orton concludes
from his data on the percentage of oysters carrying larvae that
a second female phase may occur during one season, I deem
this phenomenon still more probable in the Oosterschelde. I
did not observe the sex-change in isolated oyster individuals.

A treatise on the causes of the phenomenon of sex-change

and on the nature of its regulating power is to be found in a paper by ORTON (1927 c).

Sex-chromosomes are apparently not all-powerful in oysters. As regards the intermediate factor between sex-chromosomes and sex-determination Orton is inclined to think in the first place of sex-hormones. The presence of sex-hormones has not yet been established in oysters or allied animals (KOLLER, 1938).

ORTON suggests a connection between metabolism and sex. He suggests a metabolic rhythm as an alternative to the ordinary sex-hormone theory. He believes that sex-change is brought about by a rhythmical change in the nature of metabolism. At the female stage of the oyster the protein-metabolism is considered to be predominant and the glycogen-metabolism during the male phase. An excess of unusable metabolic products, characteristic of one sex, is believed to induce a reversal of the sex-metabolism and the sex-manifestation to that of the other sex (ORTON, 1927 d). ORTON points to the predominancy of the glycogen-metabolism during autumn, which according to him induces the male phase and he thinks that the predominancy of protein in May and June induces the female phase. It should be borne in mind, however, that these maxima are of a quite different nature. The glycogen-maximum in autumn means a real increase of the glycogen content, but the protein "maximum" in June is caused by a decrease of the amount of glycogen, so that we find a higher percentage of protein, notwithstanding the fact that the amount of protein shows a decrease as well at this time (GAARDER, 1938). This metabolic rhythm is considered to be characteristic of this organism. ORTON points to the connection between metabolism and sex in bees, Cladocera, Rotifera and the influence of Sacculina on a crab. ORTON quotes in his support the following passage from one of GOLD-SCHMIDT's papers: "The action of the hormones probably calls forth a specific type of metabolism, and this is the ultimate and direct cause of the morphological differentiation of the sexes".

Unfortunately our knowledge about the changes in the chemical composition of oysters in connection with sex-change is very limited. We know (RUSSELL, 1923, GAARDER, 1938) that the dry weight of the adult oysterbody increases during autumn simultaneously with the increase of carbo-hydrates (glycogen) during the main storage of food-reserves (fig. 5). In spring and early summer

the food reserves are largely used up in breeding, with a consequent decrease of carbo-hydrates and dry weight. The p e r c e n t a g e of proteïn shows a maximum when the oyster is in a poor condition, because of the using up of the glycogen-supply. Though the proteïn-p e r c e n t a g e shows a maximum at that time, the total amount of proteïn shows no increase at all. In summer, on the
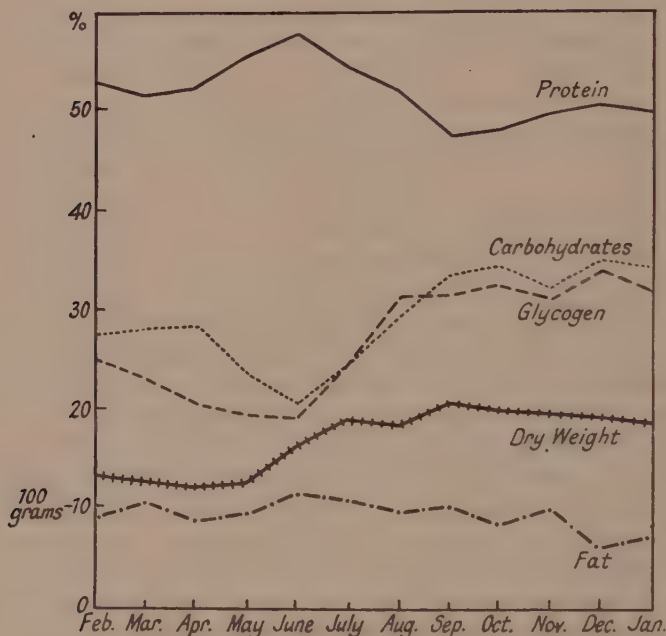
Fig. 5. Variations in the chemical composition of the oyster. After RUSSEL 1925. (Calculated on dry weight).

contrary, it shows a decrease as well, though not to the same extent as the glycogen. The production of eggs and sperm makes high demands on the metabolic processes. ORTON (1927 d) uses these data in support of his hypothesis. We should remember, however, that these analyses by RUSSELL are of oysters of u n k n o w n sex-stage, and we have to take into account the above remarks about the different nature of the two predominancies.

Considering the interference of the storage of reserves in autumn and the using up of these supplies in the next breeding season, which lead to the above-mentioned changes in chemical composition, a phenomenon that will no doubt be found in allied genera of Molluscs which show no sex-change at all, I do not believe that these analyses can be used in support of ORTON's theory.

GAARDER's analyses (1938) show a sharp decrease of glycogen-content and a decrease of the proteïn-content, but an increase of the proteïn-percentage during the breeding season. The oysters GAARDER used were of exactly the same age and had lived before the beginning of the sampling under exactly the same conditions. This led to the remarkable fact that practically all his oysters showed simultaneously the same stage of sex at the beginning of this breeding-season, in casu the female one. Most probably RUSSELL's oysters did not show the same sex-stage simultaneously, nor did the oysters (*Ostrea circumpicta*) of OKAZAKI and KOBAYASHI (1929). Nevertheless both these groups show exactly the same decrease of glycogen and dry weight during the breeding-season as that of GAARDER. I am inclined to think that the preparation of the functional male stage will result likewise in a decrease of glycogen. Perhaps the demands on the food-supplies are not *so* high as during the preparation of a female spawning, but I do not see the possibility of an essentially different kind of metabolism (proteïn versus glycogen) with regard to the building up of the different sex-products. I believe that, if GAARDER had also begun sampling from adult oysters that begin the reproduction season in the male phase, he would have obtained the same results as he actually did with his female-oysters. I should prefer a frequent sampling during the breeding season from two groups of oysters, laid out, if possible, in a cold locality as well as in a warmer one, to study the influence of the rate of sex-change. One of these groups should consist of female oysters, the other of oysters beginning the season in the male phase. Only a difference between such groups would point to other types of metabolism during the male and female period. As yet I don't believe we are justified in considering the male phase as a kind of famished female phase (GAARDER, 1938); male oysters in the beginning of the breeding season do not look like famished oysters. GAARDER's oysters lived under unfavourable circumstances as regards nourishment during the

season of reproduction; many of them died towards the end of the summer. I do not reject the idea that there may be some chemical influence which governs sex-change as an intermediate factor, but I should prefer to think of a kind of hormonal cycle and not of an influence of the kind and quantity of the materials stored.

Until we know more about the chemical composition of oysters in correlation with the stage of sex, the theory of a metabolic change as the causation of sex-change must be regarded as a mere hypothesis.

It was necessary for me to expatiate on the phenomenon of sex-change in *Ostrea edulis* to demonstrate the possibilities of periodicity in larval production during the season of reproduction.

When the external factors stop checking reproduction, the mature females will spawn their eggs; in other words: the new season of reproduction has begun. This first spawning brings on the first supply of oysterlarvae in the plankton after the incubation-period has passed.

Oysters which function first as males, because they are in the male phase at the moment of revival, will spawn during a certain time as males and after this change their sex and then spawn as females, which causes a second increase of oyster larvae after the incubation-time. When the external factors governing reproduction remain favourable for a considerable time, the oysters that spawned as females early in this season will show a reversion to the female sex once again after passing through the male phase, which sets in immediately after the first female spawning. These oysters will spawn as females for the second time in one season near the end of the summer. As the external influences, such as temperature and nourishment, will differ more or less in the various parts of an oyster-ground, it is self-evident that the above-mentioned spawning- groups will overlap one another more or less.

What I wanted to bring out is that external factors governing in a direct way the periodicity of spawning only affect oysters which contain at that particular moment mature sex-products and that the occurrence of mature sex-products is dependent on the cycle of sex-change. The rate at which this cycle is completed cannot be ascertained till we know more about the influence of temperature and nourishment on sex-change. So the influences

considered by me as internal ones are in their turn dependent on external factors, such as temperature and possibly nourishment.

It is self-evident that we are as yet unable to forecast how the spawning will be distributed over the entire breeding-season. Even when we know the external factors directly governing the periodicity of spawning, we can forecast only approximately the moment at which the first spawning in the new season will take place, but we shall not know beforehand when further spawning may be expected during the time that external factors remain favourable, until we know more of the rhythm of sex-change in *Ostrea edulis*. It will be impossible to attain this aim without lengthy and large-scale operations.

Before we pass on to a discussion of the external factors governing directly the periodicity of spawning, it will be interesting to have a look at the phenomenon of sex-change in other species of oysters. Of course we are especially interested in the behaviour of *Ostrea lurida*, an incubatory species closely related to our *Ostrea edulis*.

Ostrea lurida.

STAFFORD (1913) made the first observations on the hermaphroditism of *Ostrea lurida*. He noticed oysters containing male reproductive elements as well as female features. STAFFORD was not aware of the sex-change phenomenon in *Ostrea lurida*. It was COE (1931, 1932 c) who thoroughly investigated the sexual rhythm in *Ostrea lurida*. A fairly frequent sampling, beginning with very young oysters, clearly showed the development of the gonad, its first functioning and the sex-change cycle. COE worked at la Jolla in South-California, the southern part of the range of *Ostrea lurida*, where propagation continues for about seven months. COE could determine exactly how old his oysters were; he collected them on his concrete experimental blocks laid out in the sea at regular intervals for the sake of studying sedentary marine organisms (COE, 1932 b, COE and ALLEN, 1937). At an age of eight weeks the first signs of the development of the gonad could be observed. At about fifteen weeks the presence of the primitive ovogonia as well as the spermatogonia can be shown. The spermatogonia proliferate more rapidly than the ovogonia. *Ostrea lurida* is always protandric. Proliferation of the ovogonia and the formation of ovocytes begins before the extrusion of the

spermballs takes place. When the sperm is discharged (at an age of about five months) the oyster is in the female phase. The ovocytes build up their yolk material in the secondary gonad, the ovarian tubules, which intrude in the underlying connective tissue. At an age of about six months the first eggspawning occurs in South-California. During the incubation-period the new sperm-development can be observed. At the time of swarming the oyster is ready again to function as male and this time we shall find far more spermballs in the oyster than during the first male period. After the second male period COE noticed a period of rest, the recuparation period, during which the oyster restores its food-supplies. The duration of this period is probably dependent on metabolic conditions. If the external conditions remain favourable a second female stage occurs, after which a third male period will follow. COE is inclined to think that in colder regions the rhythm is likely to be less rapid. Perhaps we may expect an annual or even a biennal rhythm there. The alternation will stagnate when the temperature has dropped too low ($\pm$ 16° C). The alternation rebegins in the next season at the phase where the oyster left off in autumn. About 25% of the oysters will be found to contain eggs simultaneously in early spring.

COE (1931) states that: "Fertilized eggs are all alike in regard to their primary sexual inheritance, with an associated heredi- tary mechanism, perhaps metabolic in nature, which is responsi- ble for the rhythmical alternation of the sexual phase".

External conditions cannot upset the sexual alternation, but they may retard or quicken the various stages and affect the quan- tity of gametes produced. From the moment that external condi- tions allow reproduction to begin, early in the new season, the sex-change will govern the periodicity of spawning in *Ostrea lurida*. *Ostrea lurida* reaches the mature stage at an earlier age and shows (at least in South-California) a more rapid completion of the cycle than *Ostrea edulis*, but other important differences are not yet known to us.

Ostrea virginica.

Like other kinds of non-incubatory oysters *Ostrea virginica* was considered to be dioecious until quite recently.

Although many years ago STAFFORD (1913) found indications that this American oyster is protandric on the Canadian coast,

it was Burkenroad (1931, 1937) who, carrying out his investigations in Louisiana, provided us with data on the correlation of size and sex in this kind of oyster. The smaller the oyster, the higher the percentage of males. (E.g.: < 20 mm 50 ♂ — 5 ♀, < 40 mm 220 ♂ — 58 ♀, > 40 mm 151 ♂ — 315 ♀, < 80 mm 7 ♂ — 48 ♀). If we reject a differential growth-rate or a differential death-rate, this important difference in sex-percentages must indicate that sex-change is likely to occur, with a strong tendency towards protandry. It was Miss Needler (1932 b, c) who isolated oysters of a known sex and examined them in the next season. Though but few of her oysters survived, a few of her initially male oysters proved to be female in the next year and a few initially female oysters showed a male gonad. These experiments actually proved that American Atlantic oysters change their sex. In order to determine more precisely the sequence of this change in sex and the histological activities which accompany them Coe (1932 a, 1932 d) examined the gonads of a large number of oysters at frequent intervals during the first two years of their life. His histological studies were made from serial sections. Coe describes the development of the primary bisexual gonad in young oysters, which is transformed into a spermary in the vast majority of individuals. Nevertheless one year old females may be found. Coe proved that these females had not functioned as males before; they had only shown an abortive male phase which precedes the transition of the intersexual gland into an ovary. 3% to 30% of the individuals in different localities may be such one year old females. Sex-change may take place in the interval between two breeding-seasons. Sex-change appears to be more or less facultative in *Ostrea virginica*, for it is an established fact that in at least some individuals the same sex-phase may be retained for several years. Coe assumes the possibility of two genetically distinct types of males, true males and protandric males, the latter changing into females later on.

Although evidence concerning the sequence of sex-change in the extreme southern part of the range of this oyster is insufficient, it seems very probable that sex-change in *Ostrea virginica* takes place far less frequently and less automatically than in *Ostrea lurida* and *Ostrea edulis*. This difference in frequency will have a marked influence on the periodicity of spawning. As a great many oysters will show the same stages of development of

the gonads at the beginning of the breeding-season, we shall not find the same degree of overlapping of the various stages of development as in species of oysters with a more frequent sex-change. This implies the possibility of a simultaneous spawning of the vast majority of females in *Ostrea virginica*, which is quite unlike the continuous spawning (albeit with certain maxima) we find in *Ostrea lurida* and *Ostrea edulis* in consequence of their frequent sex-change. GALTSOFF (HIGGINS, 1938) has been continuing sex-change experiments with *Ostrea virginica*. He eliminates the unknown effect of injury (unavoidable when the boring device is used) by using a method which consists in determining the sex of the oyster by inducing ovulation or ejaculation by increased temperature and chemical stimulation.

Ostrea gigas.

Just like other non-incubatory species of oysters *Ostrea gigas* was formerly regarded as dioecius. AMEMIYA (1928 a, 1929) demonstrated the occurrence of a sex-change in this kind of oyster. He used the boring device, separated the oysters according to the sex-stages, isolated them in cages in the sea and examined them again after about a year. The change from male to female as well as the change from female to male was demonstrated in this way. AMEMIYA obtained no data on the frequency of the change in this manner. It is remarkable that the idea of a sex-change cycle or of a certain rhythm of alternation did not occur to this investigator. Though AMEMIYA has not adduced proofs so far, he is inclined to think that "at the beginning of every new spawning season the sex differentiates independently to the sex of the preceding season, so that the sex-change, if it appears, occurs only once in the season or in a year at the very stage when the gonad differentiates sexually". AMEMIYA does not assume protandry in *Ostrea gigas*.

As far as I can see the experiments on *Ostrea gigas* have not yet shown the latter to differ essentially from other kinds of oysters as regards sex-conditions. Until we know more about this I am inclined to assume the occurrence of a sex-change cycle.

VIII. EXTERNAL FACTORS GOVERNING THE PERIODICITY OF SPAWNING

Ostrea edulis

The external factors which have or may have a direct in-

fluence on the periodicity of spawning in oysters can be divided in biotic and a-biotic ones.

A-biotic factors

It is an indisputable fact that *temperature* is a very important factor in the biology of *Ostrea edulis*. The regulation of reproduction is to a high degree dependent on temperature conditions, the latter determining in an indirect way the periodicity of spawning during the season of reproduction by governing the frequency of sex-change and by regulating in a direct way the beginning and the end of the breeding season.

The moment at which spawning, that is to say the extrusion of the eggs into the mantle-chamber, occurs in *Ostrea edulis* can be determined by opening fairly large samples of oysters at regular intervals.

Moreover ERDMANN (1934) succeeded in observing spawning in a direct way without killing the oyster. ERDMANN noticed that oysters, placed in aquaria, will spill a few eggs in the spawning-act. In this way he could determine exactly when his oysters spawned, be it under artificial conditions.

ORTON (1920) states that a temperature stimulus of some kind is the normal impulse inducing sexual activity in marine animals, normal biological conditions being assumed. European investigators are agreed on the temperature at which spawning starts in *Ostrea edulis*. The oyster begins to breed at about 15° C and continues to breed as long as the temperature remains above that figure (ORTON, 1920, 1926, 1936, 1937 a, HAGMEIER, 1931, 1932).

This does not imply, however, that spawning always begins at the moment at which this temperature is reached! The course of the temperature during the weeks that precede this moment is of the greatest importance.

Development of the eggs starts probably at about 10° to 12° C (ORTON, 1927 b, HAGMEIER, 1931), and the time required for the maturation of the eggs is most probably mainly dependent on temperature conditions. So the maturation-period of the eggs is a function of time and temperature. If the temperature increases rapidly after a cold spring, we cannot expect maturation to be completed already at the moment when 15° C is reached. Indeed we shall find the first spawning in the new season at a somewhat higher temperature after such a rapid increase

(SPÄRCK, 1925). On the other hand, it is possible that after a
relatively warm spring a prolonged temperature between 12°
and 15° may be the cause of a spawning before 15° C. SPÄRCK
mentions a spawning at 13° to 14° C in the Limfjord in 1921
after such a warm spring. Such data cannot be said to have
been clearly specified, however, as it remains to be seen whether
the temperatures given are actually averages or merely approxi-
mations, maxima and minima not being stated, so that it is
difficult for us to judge of the exactness of the conclusions arrived
at. MAZZARELLI (1924), on the other hand, did state maxima
and minima of the temperature of the bottom-water in the lake
of Fusaro (Naples), recorded by a thermograph. His observations
make it clear that a considerable spawning is certainly possible
there before the maximum temperature reaches 15° C. (N.B.
Observations on *Ostrea lurida* (HOPKINS, 1937) have shown that
minimum temperatures are the most important with regard to
spawning).

ORTON (1927 b) criticizes SPÄRCK's methods of temperature-
recording to defend his own 15° C limit, but none the less he
states that egg-development is a function of time and tempera-
ture! I believe that, this being so, it will certainly render spawning
below 15° C possible, it being assumed that 15° C is the a v e r a g e
beginning-temperature. Both HAGMEIER (1932) and VOISIN
(1933) assume that the maturation of the eggs requires a certain
amount of warmth and so is a function of time and temperature.
In a recent paper (1936) ORTON admits the probability of the
influence of a time-caloric period. The degree at which spawning
starts is dependent on the previous course of the temperature
and will usually be about 15° C. Of course the oysters that spawn
first in the new season are those which have hibernated in an
advanced female stage and among these the individuals living
under particularly favourable temperature conditions will be the
very first. ORTON (1928 a) states that the oysters that spawn
first of all are to be found in shallow water, which shows an
increase of temperature at an earlier moment. The majority of
the oysters mature and spawn in the course of the spawning
season.

Lengthy and large-scale operations may make it possible to
obtain exact data on the nature of this time-caloric-period,
which will enable us to forecast the dates at which spawning
begins, if the temperature-conditions at the oyster beds are

known precisely. Though this would be interesting, it will never be of such great practical importance as it is in the case of the non-incubatory *Ostrea virginica*. I shall explain below that the beginning of the spawning period of *Ostrea edulis* holds no clue as to the dates at which a maximum of setting may be expected.

Though the periodicity of the swarming of *Ostrea edulis* has been frequently subjected to investigation, especially in France, and the correlation of this periodicity with external conditions has often been studied, there exist but very few papers on the periodicity of the spawning of *Ostrea edulis* during the season of reproduction. Such investigations by means of collecting samples of oysters at regular intervals during the entire season have only been carried out by ORTON, chiefly during 1925 and 1927. ORTON nowhere mentions a *direct* correlation of the periodicity of spawning with the *actual* water-temperature during the spawning season. Such a direct correlation is hardly to be expected, for we know that sex-change governs the periodicity of egg-maturation in the course of the spawning season.

Just as temperature determines the beginning of spawning, it will regulate its end in autumn. We have not many data at our disposal about the end of the breeding season. A gradual decrease of the percentage of oysters carrying young is often observed near the end of the spawning season, often before the temperature drops below 15° C. It may be that a fall of the temperature below the same 15° C level will check reproduction. I believe that the beginning of this interruption in the breeding-process does not set in at a well-defined limit. ORTON's graphs (1936) show that a few oysters, carrying young, may still be found a considerable time after the fall of the water-temperature below the 15° figure, e.g. in the beginning of November 1927. I am in a position to confirm these data by ORTON. In the Zeeland waters I noticed a very occasional gravid specimen long after a fall of the temperature below 12° C; e.g. 14 October 1937, 11 November 1937, 21 November 1938, the last one even with white embryos. The black-sick oyster, found 11 November 1937 contained living larvae of a normal size.

A close study of the diagrams will show the occurrence of an undeniable decrease in the intensity of the swarming activities near the end of the season of reproduction, in spite of the fact that the water-temperature still remains favourable or at least

rather favourable. This phenomenon of "weariness" may perhaps be attributed to a protraction of the period of recuperation, which period elapses between a phase of male spawning and the subsequent female reproductive phase. Metabolic conditions are probably among the most important factors governing the length of the period of recuperation.

MAZZARELLI (1924) carried out his investigations in the lake of Fusaro near Naples, where spawning often starts in March already, attaining its optimum in the months of April and May. After this the intensity of spawning tends to decrease, although temperature conditions remain favourable. A small percentage of spawning oysters will be found there till late in the summer season. So the longer the season of reproduction, the more conspicuous the phenomenon of "weariness" near the end of the season will be.

If no investigator advocates a direct correlation between the temperature curve and the occurrence of certain maxima in spawning in the course of the breeding season, cannot there be other a-biotic factors governing the periodicity of spawning?

Changes in salinity have never been mentioned in connection with this periodicity, at least not when they remain within normal bounds (VOISIN 1933).

A very remarkable paper is that by ORTON (1926) on lunar periodicity in the spawning of oysters. During the breeding season in 1925 ORTON examined weekly samples of 100 Fal-Estuary oysters of no less a size than about 2 inches. The ages of these oysters were not exactly known and it is doubtful if these samples can be considered as absolutely comparable. The percentage of oysters carrying young was recorded, whitesick oysters with embryos upwards to about 2 days old being recorded apart. No exact records of the water-temperature were made in the course of that season. ORTON states that "there is an undoubted, although slightly irregular periodicity in the spawning; the maximum spawning occurs at about the time of full moon" (figure 6).

ORTON's graph on the weekly samples shows 3 maxima in spawning during the breeding season in 1925: July 6, August 11 and September 2. The dates of full moon were July 6, August 4 and September 2. Unusually cold weather during August delayed the spawning for about a week, according to ORTON.

A simultaneous study of the spawning in „dumpy" oysters

did not show this correlation with the moonphases, however. There exist hydrographical factors which show a rhythmic variation in accordance with the tidal and lunar cycles and perhaps one or more of these factors may cause a lunar periodic-
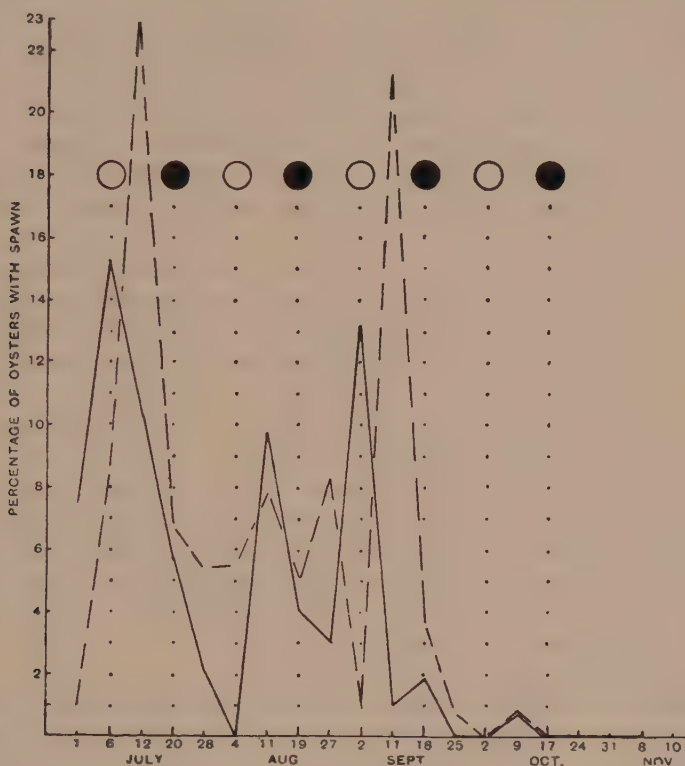
Fig. 6. Lunar periodicity in spawning. After ORTON 1926. (Continuous line graph: whitesick oysters with embryos upwards to about 2 days old; dashes: oysters carrying young more than 2 days old).

ity in spawning. ORTON declares that perhaps a thorough investigation of the local hydrographical conditions can tell us something of the nature of the influence of the moon. Nevertheless ORTON passes in review beforehand the factors which may possibly be the cause of this lunar periodicity. He thinks for example of the influence of water-pressure, which will show

extreme values during spring tides. It must be remarked that here the most extreme range of the tides occurs about two days after full-moon and after new-moon. Though full-moon spring-tides show a slightly greater range of tide than newmoon spring-tides in this part of the North-sea-coast (Admirality Tide Tables for 1925), I think it hardly conceivable that this relatively minute difference in water pressure between full-moon spring-tides and new-moon spring-tides can be the cause that spawning occurs during a full-moon spring-tide and is not observed during new-moon spring-tides. SPÄRCK (1929) never observed this lunar periodicity in the Limfjord. He thought that this might be attributed to the practical absence of tidal influences there.

Another hypothesis of ORTON is the influence of a variation in intensity and duration of moonlight, which may be thought of as an influence of the moonlight on the rate of feeding and the amount of nourishment! The absence of lunar periodicity in the Limfjord (SPÄRCK 1929) does not give support to this hypothesis, for, although there is no tidal cycle in the Limfjord, there is a moonlight-cycle!

Believers in the direct influence of the moonlight have a fine opportunity to carry out investigations in the breeding tanks, used in England for artificial propagation. In these tanks the influences of the tidal cycle have been eliminated. COLE's diagrams (1939) do not show any lunar periodicity in these tanks during 1937 and 1938, however.

Again another hypothesis of ORTON is the rapid development of the female stage round and about full moon. If, however, spawning takes place at full moon, we must expect the maturation-process to begin at least about 10 days earlier. So this influence of the full moon on the rate of development of the gonad is only conceivable in oysters which are already in an advanced female stage.

This paper (1926) makes the impression of being premature. ORTON even points to a "maximum" of spawning in October, a week after full moon: a maximum of 1 % occurring in a sample of 100 oysters! (fig. 6). As I said before, ORTON records the percentage of whitesick oysters carrying larvae upwards to about 2 days old apart. It will be clear that we are not justified in establishing periodicity in the occurrence of larvae upwards to 2 days old by means of weekly sampling. Lots of young

larvae will escape such examination and entire maxima may be overlooked.

ORTON's investigations during 1927 (1936) did not show any lunar periodicity in spawning.

American investigators like NELSON (1928 c) and PRYTHERCH (1929) assume the possibility that changes in temperature are the cause of ORTON's lunar periodicity. During springtides the tide-lands are exposed to a high degree. In fine weather the warming-up of the water will advance rapidly during springtides. But as prolonged fine weather is exceptional on the English and Dutch shores we shall seldom find a temperature curve with neat peaks at every spring-tide. ORTON gives only the air-temperatures for 1925. But it must be remembered that air temperatures do not necessarily show a direct correlation with water-temperatures.

ERDMANN (1934) observed his oysters in tanks, which spawned at any time of the day or night and at any time of the moon's cycle.

In a recent publication (1937 a) ORTON admits that he never observed this lunar periodicity again and that "this phenomenon has not yet been generally confirmed". He states that: "it seems possible that *Ostrea edulis* may tend to spawn normally at full moon or at some other definite phase of the moon in different localities, but that the regularity may be upset by local variations in seasonal conditions".

Although the periodicity of spawning in *Ostrea edulis* has not been investigated much, the periodicity of s w a r m i n g has been studied more extensively, especially in France and Holland. Although spawning and swarming are separated by the incubation-period, there is certainly some correlation between these two events. From what we know of the periodicity of swarming, lunar periodicity in ORTON's sense is out of the question, as will be discussed below. As long as lunar periodicity in the spawning of oysters has not actually been established by means of a very frequent sampling of oysters of a known age and a known past, with a precise recording of the environmental conditions and if possible by a comparison of natural conditions with the conditions in a basin, I feel inclined to regard the results obtained by ORTON in 1925 as a mere coincidence.

Biotic factors

The possibility of the influence of biotical factors on the periodicity in spawning of *Ostrea edulis* has not yet been demonstrated.

American investigators, especially GALTSOFF, showed by beautiful experiments the occurrence and importance of a stimulation of spawning by means of the sex-products of other oyster individuals, as will be discussed presently.

HAGMEIER (1931) and VOISIN (1933) point to the possibility of such a stimulation in *Ostrea edulis*, although no data to support this hypothesis are as yet at our disposal. ORTON (1937 a) states that "simultaneous spawning may occur in 15 to 20 percent of the population, so that there can be no doubt of the occurrence of a spawning stimulus". We should remember, however, that ORTON's data are based on weekly samples. So it is not certain to what degree a strictly simultaneous spawning in the sense of the American investigations may occur in *Ostrea edulis*, but I believe with ORTON that the marked spawning maxima, which are often observed in *Ostrea edulis*, may point to the occurrence of a spawning stimulus in this kind of oyster.

Ostrea lurida

COE (1932 a, 1932 b, 1932 c) informs us that spawning in *Ostrea lurida* begins at about 16° C and that the season of reproduction continues as long as the temperature is above this level; in casu at La Jolla, in South-California, for about 7 months. HORI (1933) investigated Olympia oysters introduced in Japan. He found that this oysters initiated spawning at 14° C.

These data cannot be said to have been clearly specified, however, for it is not stated whether the temperatures given are actually averages or merely approximations, maxima and minima not being stated.

HOPKINS (1937) thoroughly investigated the spawning activities of *Ostrea lurida* in the Puget Sound. As far as I know these are the most accurate and exact observations of the spawning periodicity of incubatory oysters. In the first place his hydrographical observations have been carried out very extensively. The Puget Sound shows peculiar hydrographical conditions. Most of the bays are generally rather deep and oyster culture is carried out on beds surrounded with low dikes, so that the

oysters remain submerged at low tide. On the sloping banks the diked grounds are arranged in terraces. The range of tide during a spring-tide period is about 18 to 19 feet; during neap-tides, however, the range may be only 10 or 12 feet. While the highest water-temperature is to be found within the dikes at low-tide during a period of spring-tides, the highest high-tide temperature may frequently occur in the neap-tide period, for during spring-tides the colder water of the channels in the deeper parts of the bays reaches the oystergrounds at high tide.

A correct estimate of the spawning activity of functional females was obtained by opening 100 adults two or three times weekly throughout the season on several selected typical beds. The oysters were opened on the beds to eliminate a confusion which might arise from spawning or abortion during transport. This mode of sampling has been applied in two bays during four consecutive years.

The water-temperature during the tidal cycle is subject to wide fluctuations (e.g. L.W. 28°, H.W. 18° c). HOPKINS has proved that it is certain that it is not the low-tide temperature which initiates spawning in spring, for on many days preceding the moment of spawning, the water inside the dikes reached 20 or 25° C and remained at this level for several hours. HOPKINS shows by means of fine graphs that, although maximum and average water-temperatures may be different at the moment that spawning begins in different years and in different places, the minimum-temperature will always be 12,5 to 13° C when spawning sets in. Spawning continues at a considerable rate for about six weeks in the Puget Sound. Spawning slowly increases to a maximum, then gradually diminishes until gravid individuals are found only occasionally. Sometimes there is a late secondary wave of spawning. During most years approximately 100% of the adults bear larvae (sex-change!); in 1932 a much greater spawning activity was noticed. "After the minimum water-temperature reaches the critical level for spawning, there appears to be no connection between further spawning and tides".

HOPKINS' diagrams show that there is no direct correlation between the course of the water-temperature and the amount of spawning oysters. No lunar periodicity was noticed in the Puget Sound. "Alternation of sexual phases probably is responsible for the rather slowly developed wave of spawning, for differ-

5

ent individuals are at any time in different stages of maturity".

Whether sperm will stimulate the discharge of eggs by functional females has not been demonstrated in this species, but is considered probable by HOPKINS (1937) and COE (1931).

It will be clear that we possess more adequate information about the spawning activities of *Ostrea lurida* than about those of its near relation *Ostrea edulis*. Extensive investigations of hydrographical conditions, including their rhythmical change during the tidal cycle, combined with a frequent examination of samples of comparable oysters (like those of HOPKINS), have not yet been made as far as *Ostrea edulis* is concerned. Neither species shows a close correlation between the actual water-temperature and the waves of spawning in the course of the season of reproduction, it being assumed that the temperature does not fall beneath the critical level. Sex-change determines the degree and the date of spawning in the middle of the season.

Ostrea virginica

It is easier to study the spawning activities of non-incubatory oysters like *Ostrea virginica* and *Ostrea gigas* than those of incubatory species, which spawn more secretly. A few hours after spawning an increase of oysterlarvae in the plankton can be noticed. Moreover in laboratory-experiments to determine the exact moment of spawning use is made of the occurrence of the violent valve-movements during the spawning-act. These movements can be recorded by means of a chimograph (NELSON 1922). *Ostrea virginica* requires a higher minimum-temperature to spawn than *Ostrea edulis* and *Ostrea lurida*. This critical level is 20° C. (STAFFORD, 1913, CHURCHILL, 1920, GUTSELL, 1924, NELSON, 1928 a, b, c, PRYTHERCH, 1929, GALTSOFF, 1930 a, 1932). NELSON (1928 b) complains, that "*Ostrea virginica*, the most valuable mollusc in the world[1]) is barred from most of the otherwise favourable coastlines of the earth, since the waters there rarely attain a temperature of 20° C for a sufficient period to permit spawning, while the inferior species[1]) *Ostrea lurida* and *Ostrea edulis* may thrive there, since much of the coastline of the northern hemisphere rises to 15° C, or slightly above, for the time necessary to permit these species

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[1]) Spacings are mine. So far I have never met a connoisseur of oysters knowing both *Ostrea edulis* and *Ostrea virginica* who asserted that *Ostrea virginica* is the better of the two.

to spawn". We shall see later on that although *Ostrea edulis* will spawn at these low temperatures, a setting of commercial magnitude can only be expected when the water reaches temperatures of 20° C and higher. NELSON (1921) states that the development of sex-products starts at a temperature of about 10° C and that oysters begin spawning when the temperature of the water reaches 20° C. A more detailed study of the gonad showed later on (NELSON, 1928 c) that sperm-production begins at about 10° C, deposition of yolk-material at about 15° C and the definite maturation of the eggs at about 18 to 20° C, though a wide individual variation will always be noticed. In case of a too rapid rise of temperature the critical level for spawning will be reached in advance of the maturation of the gonad (HOPKINS, 1931). Therefore it is always necessary to investigate both the development of the gonad and the phenomenon of spawning (GALTSOFF, 1934). If, in extreme cases, in the northern part of the range, the temperature never, for any length of time, attains or exceeds 20° C, the spawn may not be thrown out at all, but will be reabsorbed by the oyster (NELSON, 1921). The length of the latent period, which extends from the moment 20° C is reached till spawning, bears a definite relation to the slope of the temperature curve. NELSON showed (1927) that spawning of *Ostrea virginica* may be expected to take place approximately 160 degree-hours after the temperature of the water reaches 20° C and remains at this level. Thermograph records of the water temperatures obtained during these "latent periods" in the years 1924, 1925, and 1926 were measured by a planimeter and the area enclosed between the temperature curve and the 20° C line was replotted in the form of a smooth triangle on the same base-line of elapsed time. The areas of these three triangles show a definite relation to the length of the baseline of elapsed time. NELSON computed mathematically that, if the temperature does not rise above 20° C, spawning may be expected to occur approximately 100 hours after the temperature reaches 20° C.

Local conditions may affect the initiating of spawning. So PRYTHERCH (1929) in his study on the spawning and setting behaviour of *Ostrea virginica* near Milford, Conn., concluded that spawning begins when the temperature at high tide reaches 20° C. He considered that the lower pH (7,2) of the water at low tide, when the temperature was much higher, was the factor preventing spawning. At high tide, on the other hand,

when the water reached about 20° C and the pH was about
8,2, the oysters spawned.

Spring-tides are a factor in determining the moment at which the
20° C level is reached, owing to the warming-up of the tidal lands
during extremely low tides, seeing that the latter may cause a
rapid rise of the water-temperature (PRYTHERCH, 1929, 1934 b).

It will be clear that we can only count on a regular rise in
water-temperature during spring-tides in a region where pro-
longed fine weather prevails. On the Northsea coast e.g., with its
very changeable weatherconditions, such a constant connection be-
tween the water-temperatures and the tides is not to be expected.

GALTSOFF (1930 a, 1932) placed single oysters in tanks (30
litres), controlled factors like oxygen-percentage, pH and salin-
ity, and was able to vary the temperature with the aid of a thermoreg-
ulator. The valves of his oysters were attached to a chimograph
in order to register valve movements and spawning action.
GALTSOFF found it impossible to effect spawning by merely
raising the water-temperature as long as it remained below
24, 5°C. The latent period, which had to elapse before spawning
begins, showed wide individual fluctuations (22 to 217 minutes).
However, there appeared to be another method of initiating
spawning even below 24,5° C, but above 20° C, viz. the addition
of oyster-sperm to the water of the tank! GALTSOFF's fine experi-
ments showed that the action of sperm on a female individual
must be considered as a stimulation, for this action shows the
all-or-none principle. The minimum amount of sperm causing
stimulation proved to be about 150 sperms per cc. of water.
Addition of sperm provokes no immediate spawning, but there
must elapse a latent period of 6 to 38 minutes, independent of
the amount of sperm, before spawning begins. The duration of
the spawning-act proved to be $3\frac{1}{2}$ to 74 minutes, likewise inde-
pendent of the amount of sperm and of the duration of the latent
period. GALTSOFF found no difference between the character
of a spawning-act provoked by a mere raise of the water-
temperature (above 24,5° C) and one by sperm-stimulation
(above 20° C). The occurrence of a latent period indicates the
indirect nature of this reaction. Sperms of Mya and Mytilus
proved to be inactive and collodion filtration as well as a heating
to 55° C eliminates the stimulative power of the sperm. Below
20° C no sperm-stimulation was possible.

A single male oyster spawned at about 22 to 23° C by a mere

increase of the temperature. Addition of egg-suspension (only eggs of the same oyster-species proved to be active!) provokes a direct spawning reaction without any latent period. This agens is proof against boiling and filtration. It is remarkable that sperm-addition may also induce spawning in male oysters, but in that case a latent period has to elapse before spawning begins. GALTSOFF assumes that the influence of sperm on both males and females operates via the intestinal tract, as is indicated i.a. by the length of the latent period.

It is clear that this chemical stimulation of spawning has a great influence on the periodicity of spawning in *Ostrea virginica*. While single oysters will not spawn until a temperature of 24,5° C is reached, this stimulation may cause the simultaneous spawning of all the oysters in the neighbourhood that contain mature female sex-products at that moment. A few spawning males may initiate a wholesale spawning at any temperature above 20° C. This is the reason why investigators in various places may notice a prolific spawning on one particular day (PRYTHERCH, 1929). Of course the gonads must be at the required state of maturity before spawning will take place. Where external conditions are about the same all over an oyster bed, a simultaneous maturity of a considerable part of the stock may be expected, for no rapid sex-change interferes, as in *Ostrea edulis* and *Ostrea lurida*.

Indeed we may expect a simultaneous spawning on one particular day in such localities where the entire stock lives at an equal depth, so in about equal external conditions, e.g. in Little Egg Harbor (NELSON, 1921), Milford Harbor (PRYTHERCH, 1929) and other shallow bodies of water.

After eggspawning the individual will not respond to any sperm-stimulation for some days (refractory period). When the ripe eggs are spawned out, others pass down from the tubes of the gonad and take their place (no sex-change into the male phase immediately after female spawning in *Ostrea virginica!*), so that the spawning of an oyster may continue over a considerable part of the summer season (NELSON, 1921). So subsequent waves of spawning, likewise induced on one particular day by the action of sperm-induction, may occur when tempera-ture remains favourable. It depends on local conditions whether we can expect more than one wave of spawning. Thus NELSON (1929) states that four main broods of larvae were produced in Barnegat Bay during 1927.

The peaks in the graphs that record the amount of larvae are of-
ten found to coincide with those in the temperature curve. Owing
to the time required for the development of eggs into shelled
larvae, we shall always expect the increase in the amount of
larvae some 24 hours after the moment of spawning. Such a
correlation of temperature and spawning, though it certainly
cannot always be shown, is conceivable, seeing that the individ-
uals that spawn first of all require a mere rise in temperature
as a stimulation. This spawning will thereupon induce the
chemical stimulation, resulting in the subsequent simultaneous
spawning of those individuals which are mature at that particular
moment. According to PRYTHERCH (Long Island Sound) a
relatively light first spawning during spring tide (rapid rise in
water-temperature!) will generally be followed about two weeks
later (during the next period of spring-tides, when the water is
warmer) by a more general spawning. In other localities spawn-
ing conditions are more complicated. When the oyster beds are
not situated in shallow water, but when the oysters occur at
different depths, it is clear that these oysters do not all live under
the same temperature-conditions. In the latter case maturation
cannot be expected to occur simultaneously. Oysters in shallow
water spawn before those at a greater depth, so that in any
body of water where oysters may be found at a depth from 1
to 30 feet and more, spawning will be found to occur over a
considerable part of the spawning season. In such places spawn-
ing may be prolonged throughout the greater part of summer,
with an occasional sharp increase of short duration, e.g. in
Delaware Bay (NELSON 1921).

Summarizing we may state that there is much difference
between *Ostrea virginica* and the two incubatory species *Ostrea
lurida* and *Ostrea edulis* as regards the periodicity of spawning.
This difference is not in the first place due to the phenomenon
of incubation, but must be attributed to the less rapid and more
irregular sex-change in *Ostrea virginica*. This makes it possible
for the bulk of females to show the same stage of gonad-develop-
ment. In such cases a chemical stimulation by means of the
mutual sex-products may induce a strictly simultaneous spawn-
ing on one particular day. This will be found to be the case in
shallow bodies of water. Oysterbeds with a more irregular
bottom-configuration, and consequently with different tempera-

ture conditions, interfere with this phenomenon and will show a prolonged spawning, with an occasional sharp increase, thus resembling the spawning in *Ostrea edulis* and *Ostrea lurida*, with the difference that in this case the prolonged spawning is not due to the overlapping of the stages of sex-change, as it is in the two latter species!

It is not certain that sex-change has no influence at all on the spawning periodicity in *Ostrea virginica*, especially in the southern part of the range.

Many data on the periodicity of spawning in *Ostrea virginica* obtained both by gonad inspection and observations on the occurrence of oysterlarvae in the plankton can be found in the papers of HOPKINS (1931, Galveston Bay Tex.), NELSON (1917, 1922, 1923 b, 1924 b, 1925 a, 1926, 1927, 1928 a, 1929, 1930, 1931, 1932, New Jersey) and PRYTHERCH (1929, Milford Harbor), many of them illustrated with graphs and diagrams.

Ostrea gigas

Although we do not know very much about the natural periodicity of spawning in this non-incubatory oyster, because no extensive investigations on samples of adult oysters and on the appearance of the larvae in the plankton have been carried out, there are reasons to assume that *Ostrea gigas* acts very similarly to *Ostrea virginica* in this respect.

GALTSOFF (1930 a, 1932) found that by merely raising the water-temperature *Ostrea gigas* will spawn at about 30° C. Just as in *Ostrea virginica* the addition of sperm will induce mature females to spawn at a considerably lower temperature, in casu at about 25° C (GALTSOFF 1930 a, 1932), or perhaps even at lower temperatures (ELSEY 1933). The nature of this stimulation proved to be quite the same as of that in *Ostrea virginica*.

Very interesting is the practical application of this knowledge as described by ELSEY (1933, 1936). Japanese oysters imported into the Canadian Pacific waters near Ladysmith Harbor often fail to spawn there, owing to a too low water-temperature. Sometimes they spawn very late in the season; the result of this is that probably no suitable setting will be obtained and that the adult oysters will begin the winterseason in a bad condition, which is a disadvantage from a commercial point of view.

ELSEY placed a lot of adult oysters in live-boxes and anchored them above the oyster beds on a suitable day as regards temperature and maturity conditions.

High slack water has proved to be the right moment to set to work. The sperm of a few dozens of oysters is shed into the live-boxes. This induces these oysters to spawn. When they have all been put into action, the bottoms of the sinkfloats are opened. Favoured by the practical absence of currents at high slack water, all the oysters in the beds in the neighbourhood are soon stimulated to spawn. So this intervention may result in a good setting as well as a good quality of the adult oyster in the next winter-season.

IX. FERTILIZATION

It is fairly certain that fertilization in non-incubatory species like *Ostrea virginica* and *Ostrea gigas* takes place in the free water after spawning. Fertilization in vitro succeeds easily in these species when sperm is added to seawater containing mature eggs. Relatively little is known about fertilization in non-incubatory species. No recent investigator has advocated self-fertilization.

Ostrea edulis

HOEK (1884) assumed that fertilization takes place *before* the eggs are spawned. Though HOEK occasionally discovered sperms in the renal tubulus of the females, he could never demonstrate a functional spermatheca. HOEK never actually saw an oyster spawning, but he referred to the observations of WAALEWIJN (1885), who declared that he once saw eggs in segmentation, issuing from the genital aperture.

ORTON (1927 a) continued the studies of HOEK and searched for a spermatheca. He concluded that the precise situation of a functional spermatheca has not yet been defined. He thought it incredible that such a spermatheca, in which sufficient sperm to fertilize about a million eggs of a female oyster can be stored, will ever be found in *Ostrea edulis*. ORTON observed that sometimes a fairly considerable percentage of the eggs of *Ostrea edulis* appear to be unfertilized, which indicates that sperm has not been abundant enough at the right time.

SPÄRCK (1925) states that he once found unsegmented eggs in the mantle-chamber of an oyster. ERDMANN (1934) kept his

oysters in tanks. He could observe the exact moment at which his oysters executed their secret spawning-act, for a few eggs are always spilt by a spawning oyster, which eggs could be detected on the bottom of his tanks right under this oyster. ERDMANN concludes that the eggs are fertilized after spawning and that the sperm is brought into the mantle chamber (where the eggs are held) with the water pumped up by the gills. ERDMANN succeeded in observing microscopically the act of fertilization in eggs found on the gills immediately after spawning: "Oft konnte ich diesen Vorgang (spawning) direkt, und bei sofortiger, mikroskopischer Untersuchung sogar noch den Befruchtungsakt beobachten". Yet there have been writers in recent years (e.g. BIERRY and GOUZON, 1939, BORDE and BORDE 1938) who assume that fertilization takes place before spawning. The sperms are assumed to enter the mantle chamber with the inhalent water and thence to pass through the gill-slits into the genital aperture, which procedure would render a spermatheca superfluous. However, I have never yet come across a recent description of observations on fertilized eggs in the ducts of the gonad.

Though it has not been proved that fertilization before spawning never occurs, I am inclined to think that the difficulty in finding unfertilized and unsegmented eggs on the gills (owing to the very short duration of the unfertilized and unsegmented period after spawning) has been the main reason to assume fertilization before spawning. I am inclined to attach more value to the accurate observations of ERDMANN and SPÄRCK than to the vaguer and more speculative data of earlier writers.

In non-incubatory species the sperm will find the eggs in the free water, most probably through chemotaxis, as has been proved to be the case in so many other acts of fertilization. It would be interesting to know to what degree chemotaxis plays a part in fertilization in incubatory oysters. How does the sperm reach the mantle chamber: is it carried along with the watercurrent brought about by the action of the gills, or does it reach the chamber on its own account through mere chemical allurement? I assume that the speed at which the sperms move on is far too little for them to be able to counteract watercurrents of some importance, such as the current brought about by the action of the gills and the tidal currents in the free sea-water. So I am inclined to believe that the first part of their way to the eggs

will be covered passively, the sperms being carried along with the inhalent water. After the mantle chamber has been reached, the sperms on the latter part of their way may be guided by chemical allurement. I have to remark, however, that so far no experiments have been made to determine the rate of flow of inhalent water in oysters during incubation.

Ostrea lurida. Ostrea denselamellosa

Fertilization after spawning and the entrance of sperm with the water-current caused by the gills is assumed likewise for *Ostrea lurida* (Coe 1931, 1932 c) and *Ostrea denselamellosa* (Senô 1929).

X. INCUBATION

The eggs are held in the mantle or branchial chamber adjacent to the gills and labial palps, where in incubatory species of oysters they develop for a considerable period. It is clear that the inhalent stream of water will keep them in their place on the gills during the period of incubation.

Ostrea lurida

Stafford (1914) was the first to study the duration of the incubation period in *Ostrea lurida*. He would periodically prise the valves of a gravid specimen partly open and take a sample of the larvae. Such handling of the specimen may, however, easily cause a disturbance of the normal function and interfere with larval development. Stafford computed that the duration of the incubation period of *Ostrea lurida* in the waters of British Columbia is about 16½ days. Coe (1931) estimated that the larvae of this oyster develop normally for a period of about 10 to 12 days in Southern California.

Hopkins (1937) moreover used the data yielded by his frequent samplings of oysters during several seasons of reproduction to fix the duration of the period of incubation. As a single brood was found to consist of larvae at approximately the same stage, within relatively narrow limits, Hopkins found it possible to organize the results in such a way that the gravid specimens, bearing broods at the same stage of development, could be grouped and thus followed through the various stages, as in subsequent samples one group would continue to recur until the larvae reached the

size at which they are discharged. HOPKINS concluded that the normal duration of the incubation period is about 9 to 11 days, which accords with COE's estimate (1931). STAFFORD's 16½ days' period may be correct for the locality in which *he* worked, or it may due to his method of analysis.

Ostrea edulis

What do we know about the duration of the incubation period in *Ostrea edulis*?

ORTON (1926, 1936, 1937 a) estimated that under natural conditions oyster larvae are retained in the mantle cavity for a period of about 7 to 10 days from the date of spawning. He founded this estimate on a comparison of his graphs on the percentage of oysters containing white and conchiferous larvae. We should remember, however, that ORTON sampled only once a week. So HOPKINS (1937) is quite right when he states: "Apparently his samples were not taken with sufficient frequency to permit analysis in the manner described above. Nevertheless it is probable that the period of larval development within the maternal brood chamber is not greatly different in the two species".

Further information was obtained partly by isolating individuals carrying embryos or larvae at a known stage and observing the subsequent stages by opening the shells or inducing the oyster to throw out his larvae and partly by taking embryos and larvae from the parent and keeping them in dishes at known temperatures; but development tends to become irregular under artificial conditions. ORTON states (1936): "The whitesick stage is thus normally of about 3 to 3½ days duration, the grey-shelled stage about 1½ to 2 days or less, and the black-sick stage of variable duration, probably 4 days or less".

SPÄRCK (1925) collected some data on the duration of incubation during his regular gonad-examinations of oysters which were kept in tanks in his sex-change experiments. Though, apparently, his oysters did not live under natural conditions, he concluded that the first part of the development (till the black-sick stage) requires about 3 to 4 days and that the second part of incubation requires about 5 to 6 days at a temperature of 14° to 16° C and about 2 days at an temperature of 18° to 20° C.

ERDMANN (1934) likewise kept his oysters in tanks under

controlled temperature-conditions. At a temperature of about 13° to 14° C his oysters proved to incubate the larvae for about 18 days. At 17° to 18° C for about 14 days, and at 23° C for about 6 to 8 days. ERDMANN stated that low temperature will not only delay swarming to the above-mentioned extent, but that it will also cause these larvae to show larger dimensions and a more advanced stage of development at the moment they are discharged. If this phenomenon occurs regularly in nature, continued bad weather will cause a delayed swarming and an increasing size of the freshly spawned larvae with a decreasing water-temperature. We shall see in the next chapters in how far, under natural conditions, this assumption corresponds with the facts.

XI. THE PERIODICITY OF SWARMING

STAFFORD used the word "swarming" to designate the final release of the larvae from the maternal brood-chamber in contrast with the original spawning whereby the eggs are released from the gonad. It has been remarked before that swarming is only a delayed completion of the spawning process.

Ostrea lurida

The Olympia oyster has not yet been taken in the very act of swarming. Even HOPKINS (1937) declares that he does not know whether the discharge of the larvae is accomplished in the same manner as the discharge of the eggs in oviparous oysters. As HOPKINS observed that during abortion the embryos are forcibly ejected by violent shell movements, he is inclined to believe that natural swarming takes place in about the same manner.

The assumption of BONNOT (1936), who presumes that the larvae leave the mantle cavity of the adult in the outgoing water current, must be wrong, for this would require a passage of the developed larvae through the gill-slits, as the outgoing water-current comes from the exhalent chamber (suprabranchial = cloacal chamber) and the larvae develop in the inhalent chamber (= branchial chamber). The amount of larvae of Ostrea lurida in the water has not been determined quantitatively. Observation of the increase of young oysterlarvae in the water is the only exact way to determine directly the rate of swarming. Now HOPKINS computed the periodicity of swarming from the

graphs on the percentages of oysters incubating larvae, so that his data are indirect ones.

As changes in the percentage of oysters carrying larvae in an advanced stage of development may be attributed to losses owing to swarming as well as to gains owing to a further development of larvae of younger stages, it is always difficult to estimate the actual extent of swarming only from such graphs on the percentage of oysters carrying larvae.

I did not come across any discussion of a possible influence of external conditions on the rate of swarming in *Ostrea lurida*.

Ostrea edulis

As far as I know only ERDMANN (1934) has described observations of the act of swarming in *Ostrea edulis*. His swarming oysters, kept in tanks, opened their shells widely, and then ejected a cloud of larvae by a violent contraction of the adductor muscle. This action was repeated several times, with longer or shorter intervals. The entire swarming-act may be accomplished in a few hours. ERDMANN states that he made this observation more than once. Although this action is essentially the same as that by which oviparous oysters discharge their eggs, there is some difference in the rate at which it is accomplished. Instead of a succession of rapid rhythmical closing movements, as can be observed in *Ostrea virginica* (NELSON, 1922), ERDMANN observed several intermittent violent shell-movements, spread over a fairly considerable lapse of time.

Indirectly some figures about the periodicity of swarming can be computed from data on the percentage of oysters carrying larvae. ORTON's graphs on such data about *Ostrea edulis* (1926, 1936) make an approximate computation even more difficult and less reliable than the same kind of data from HOPKINS' investigations on *Ostrea lurida* (1937), because of ORTON's less frequent sampling. ORTON does not mention any direct influence of external conditions on the moment of swarming. He confines himself to the statement (1937 a) that the larvae are retained longer in cooler and shot out earlier in warmer conditions, which may be understood from the influence of temperature on the duration of the period of incubation, as has been discussed before.

Figures about the periodicity of swarming in *Ostrea edulis* can be obtained directly by means of quantitative and semi-quantita-

tive studies on the amount of oysterlarvae in the plankton. PETERSEN (1908) stated that oysterlarvae can be found in the water already at 15° C. German investigators (KÄNDLER, 1928, HAGMEIER and SCHUBERT, 1930) made some observations on the amount of oyster larvae in the plankton after the sowing-out of a certain amount of Dutch seed-oysters near List in the Wattenmeer. Their method of sampling can hardly be called quantitative. Detritus often stopped up the meshes of their plankton-net. Moreover the sampling took place only a few times during the season, with fairly long intervals. After swarming they noticed many young larvae in the neighbourhood of the sown-out mother-oysters. An observation on a profuse swarming shortly after a period of stormy weather led to the conclusion that unfavourable weather prevents swarming and that the mother-oysters await more favourable circumstances before they eject their larvae. In 1931 and 1932 we still find HAGMEIER expressing the same opinion: "Es findet dann, wie dies auch von KÄNDLER im Freien beobachtet wurde, beim ersten Eintritt günstiger Verhältnisse ein massenhaftes Ausstoszen von Brut statt".

I do not believe that a single observation on swarming after a period of bad weather justifies the conclusion that oysters await favourable weather to eject their larvae. More frequent and better quantitative sampling is necessary if we want to obtain adequate information justifying such a conclusion.

More or less frequent sampling of plankton has been carried out in the most important French centres of oyster-production since 1921, with a view to forecasting the setting. Appreciation should be expressed for the inestimable initiative of the French investigators, who have been the first in Europe to try to build up a regular advisory service in order to render assistance to the oyster farmers in raising spat-production. LAMBERT (1935) describes the development and the methods of these investigations. Many publications of these investigations contain graphs showing the amount of oyster larvae in the plankton. Water-temperature and salinity are recorded likewise: LEENHARDT (1924), BOURY (1928, 1929 a, 1930), RAPHENNE (1931), VOISIN (1931, 1932), HERMAN (1935, 1936, 1937, 1938 a, 1938 b), BORDE (1929, 1930, 1932, 1935, 1936, 1937), LADOUCE (1938 a, b). (The latter two at Arcachon, the former at le Morbihan). I have already described the methods used by these French

investigators in a previous chapter. I have stated that the towing of a plankton-net cannot be considered an ideal quantitative method of sampling, and it is especially unsatisfactory for the special reason that it does not enable us to estimate, even approximately, the volume of filtered water. Many of these graphs on the amount of larvae show a very steep course.

It is noteworthy that such a steep course appears especially in those graphs that reflect the greatest frequency of sampling. The less frequent the sampling, the more quiet the course of the graphs generally is.

So the graphs of LEENHARDT (1924), for instance, are "quiet" probably mainly in consequence of infrequent sampling.

An extreme of the other kind is presented by the steep graphs on the larvae in the river d'Auray in 1935 (HERMAN, 1936). A steep course does not necessarily mean, however, that the method of sampling is quite unsuitable, for graphs on really quantitative samples may show such a steep course as well.

When we collect plankton-samples right above the place where the larvae-producing mother-oysters live, we may expect to find enormous amounts of young larvae during swarming. A sample taken a few days later in the same place, under the same tidal conditions, may contain but a comparatively small fraction of such an amount. This does not point to an enormous mortality, but it only means that the young larvae are distributed more uniformly over a larger volume of water now. It is clear that a graph on the number of larvae per unit of water near the oyster beds will show a much steeper course than a graph on samples taken at some distance from the place where the young larvae are set free.

Consequently very steep graphs may be due to the local occurrence of larvae that have been liberated very recently as well as to an unreliable method of sampling. Larvae which have been in the plankton already for several days, however, will be distributed more equally than these newcomers. So if we find that a graph on the amount of older larvae in the same water is likewise very steep, our method of sampling is most probably not quantitative. When, however, the amount of older larvae remains fairly constant in the daily samples, coinciding with enormous variations in the amount of young larvae, we may conclude that our method of sampling is all right and that swarming takes place.

It is a pity that the graphs of the French investigators indicate only the total amount of larvae in the samples, so that we cannot see from these graphs the degree of variation in the amount of older larvae; consequently there is no possibility of checking the reliability of their method of sampling.

Partly the steep graphs will no doubt be caused by the proximity of adults ejecting young larvae. A less steep graph may be the result of sampling in a place fairly remote from the swarming centre as well as of a less frequent sampling! That such steep graphs do not necessarily point to a tremendous mortality, but that they may be due to a dispersion of the newly-liberated larvae, is not always realized by the French investigators. So BOURY (1928) declares: "De l'existence des variations assez brutales dans les quantités de larves nageantes, on déduit que celles-ci périssent en grand nombre".

In their commentaries on the graphs the French authors sometimes declare that the peaks of the temperature-course coincide with peaks in the course of the amount of larvae, from which they concluded a direct influence of the temperature on the rate of swarming (BORDE, 1935, 1936, LEENHARDT, 1924). LEENHARDT even states that swarming is a direct function of temperature: "Nous disions (1922) que l'huître peut pondre à partir de 15° (si les mères ont suffisamment incubé); nous devons ajouter que la ponte est fonction de la température, et subit, à peu de chose près, les mêmes variations. Le nombre de larves libres augmente avec la température". VOISIN (1933) still states that no considerable emission of larvae will take place below 18° C.

A close study of the graphs in all these papers will make it clear, however, that a correlation between the actual water-temperature and the periodicity of swarming certainly does not exist! Though sometimes a considerable swarming will be observed during high water-temperatures, there are a great many data from observations of swarming at much lower temperatures, even below 18° (BOURY, 1928; Cuhan en '27). So LEENHARDT's conclusion has not been confirmed by the numerous data from subsequent French investigations.

Holland

In the course of my investigations in recent years I have obtained much information about the periodicity of swarming in the

Oosterschelde. The frequent sampling (daily at the height of the season), at the same stage of the tide, of known quantities of water make it possible to get a good impression of the amount of oyster larvae in the two places where the samples are collected. At first I used to record my data in the form of a graph on the total amount of larvae in 100 litres. There soon appeared to be a marked difference in character between our two sampling-stations. Though the average number of larvae did not differ very much, the two graphs differed greatly in the steepness of their course.

The reason of this will be clear, for the first station (Yersche Bank) is situated in the middle of the most important oyster-beds of the Oosterschelde and may be considered to represent the centre of larvae-production, while the second station (Kattendijke) is situated fairly remote from the important oysterbanks, and receives its larvae by the tidal movements. At the first station the occurrence of swarming will be observed immediately. On days of swarming we shall notice high peaks in the course of our graphs, while a rapid decline may be found the next day owing to the dispersion of these young larvae through a larger volume of water. At the second station,
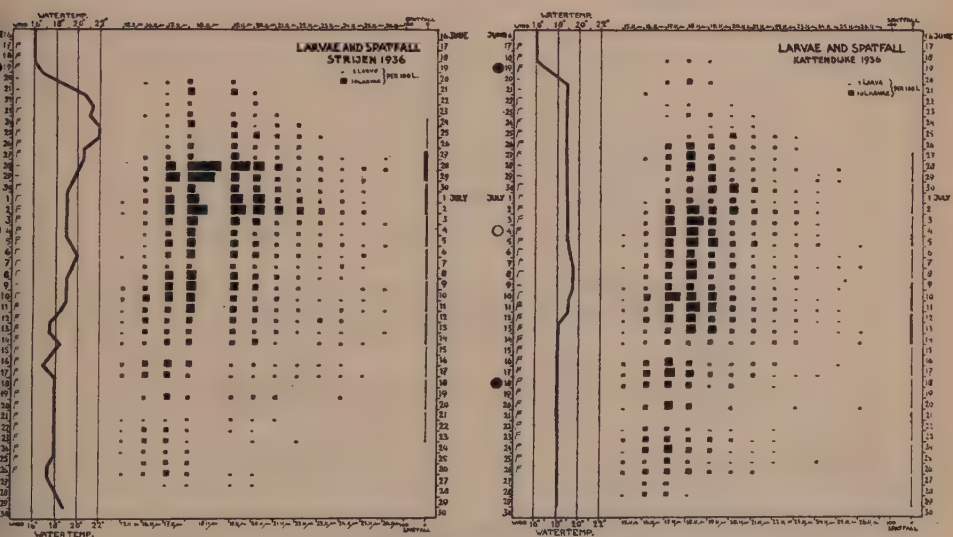
Fig. 7. Larvae and spatfall. Strijen and Kattendijke 1936.

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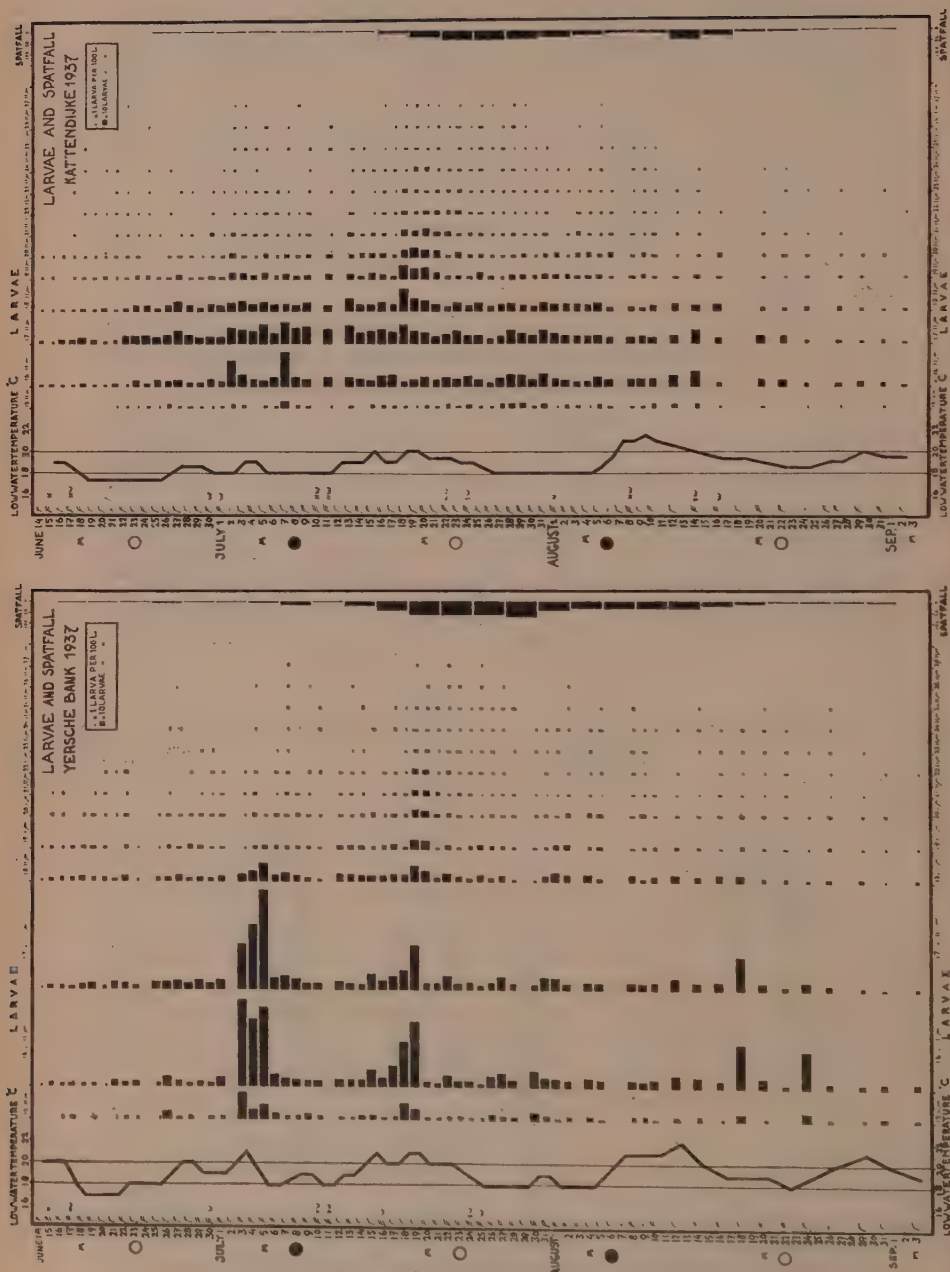
Fig. 8. Larvae and spatfall Yersche Bank and Kattendijke 1937.
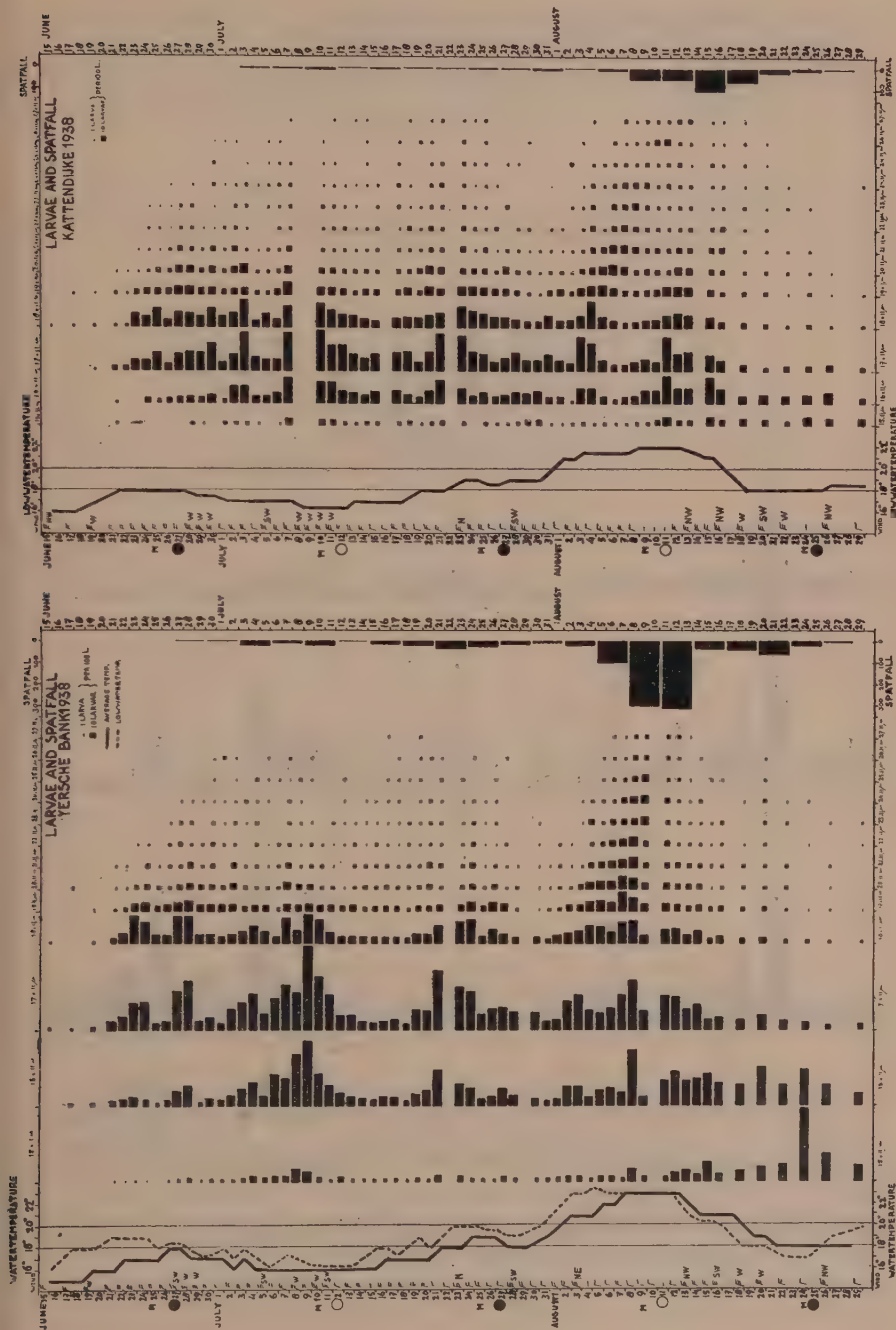
Fig. 9. Larvae and spatfall Yersche Bank and Kattendijke 1938.

however, things take place more gradually. We shall seldom observe sharp increases there. The number of larvae will rise fairly gradually after days on which swarming of some importance has taken place and decreases in the number of larvae
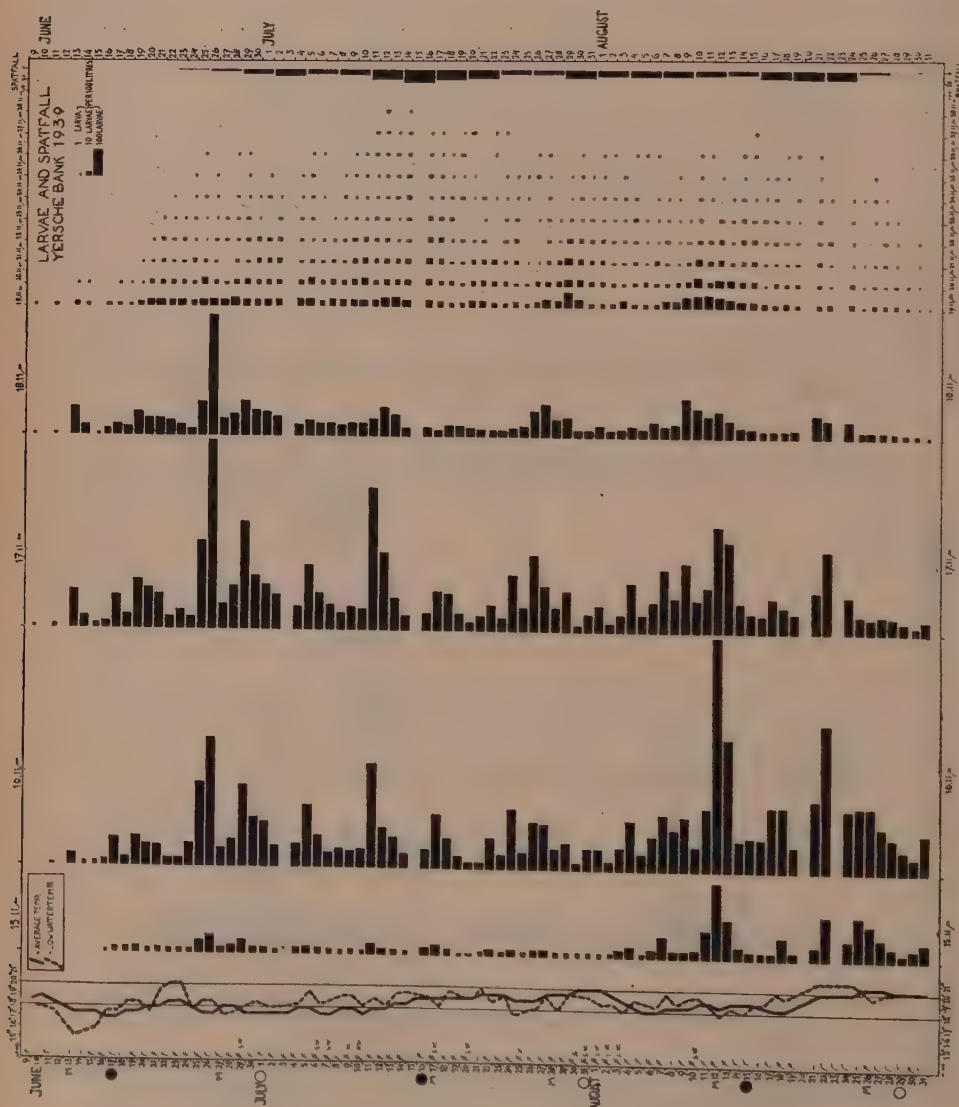
Fig. 10a. Larvae and spatfall Yersche Bank 1939.

will also take place gradually. It will be clear that we shall have to focus our attention on the data of the centre of larvae-production, in studying the periodicity of swarming.

These data are exhibited in the figures 7, 8, 9, 10.
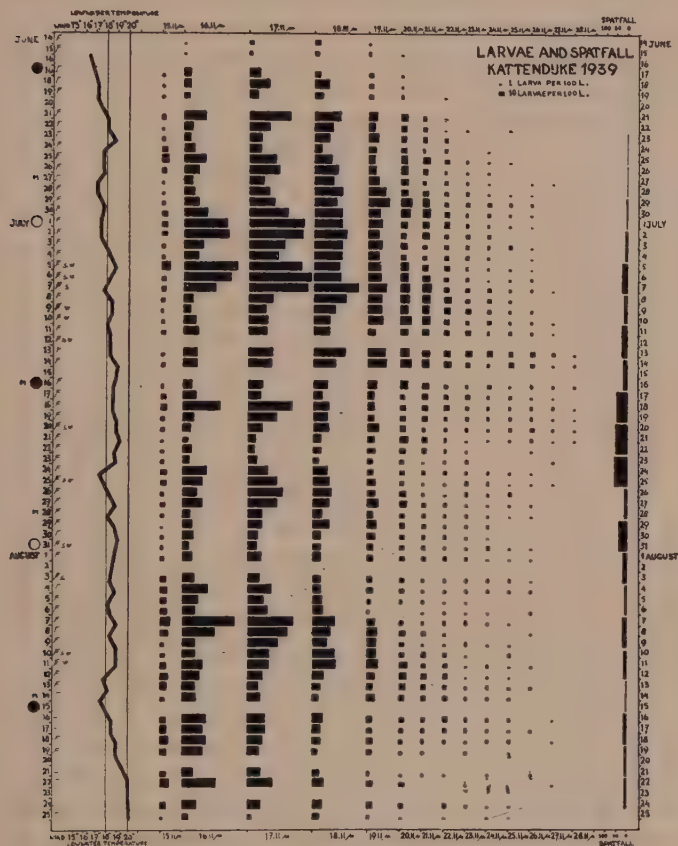
Fig. 10*b*.   Larvae and spatfall Kattendijke 1939.

I succeeded in finding a kind of diagram that made it possible to record the number of larvae per unit of water, the size of these larvae, the setting of spat, and some a-biotic factors, such as water-temperature, wind and the phases of the moon. I devided the larvae in groups according to the size of their shells. One degree of my micrometer corresponds with 11 μ in the combination of lenses used, so each group consists of

larvae 11 μ larger on an average than those of the preceding group. The diagram on the number of larvae at the Yersche Bank (centre of larvae-production) shows many marked increases in the number of young larvae, which points to swarming. The swarming is not limited to a few days, but is distributed over several weeks, showing more and less important peaks.

I want to point out, however, that we are not justified in deducing the absolute value of swarming directly from the amplitude of the number of newly-liberated larvae in the diagrams. The degree of proximity of the liberating adults will not always be the same. When we want to compare the values of swarming we must consider likewise the number of larvae during the next days and the subsequent increase of larvae in a more distant place, like Kattendijke.

These diagrams do not reveal any marked direct correlation between the periodicity of swarming and the course of temperature. Sometimes intensive swarming occurs during a high water-temperature (e.g. July 3, 4, 5: 1937, July 18, 19: 1937, early in August 1937); sometimes during an increase of the temperature (e.g. August 18: 1937) and sometimes during relatively low water-temperatures, even below 18° C (e.g. July 8, 9: 1938). I want to emphasize the heavy swarming during the days early in July 1938, when the mean water-temperature (recorded by a thermograph) was 16° C and the maximum temperature during low-water did not reach 18° C! This important swarming under these temperature conditions proves that the opinion of LEENHARDT (1924) and VOISIN (1933), that no important swarming will be observed at lower temperatures, does not hold good for the Oosterschelde. MAZZARELLI (1924) stated likewise that considerable swarming may occur before the water reaches 18° C. The view of the German investigators, who assume that the young larvae are held in the mantle-chamber till the weatherconditions are more favourable, does not hold good either. For not only did low water-temperatures occur during swarming in July 1938, but also stormy weather.

It is my belief that swarming will take place when the larvae have been sufficiently incubated. So the periodicity in swarming is mainly governed by the periodicity in spawning.

The period of incubation may be protracted somewhat by lower and shortened somewhat by higher water-temperatures, but as the influence of temperature on the periodicity in

spawning is rather complex, the periodicity in swarming cannot possibly be a simple function of the actual water-temperature.

There is *no direct correlation* between the dates of swarming and factors like the actual water-temperature, the strength of the wind, rain, air-pressure, etc. The salinity varies but very little in the Oosterschelde and as these variations do not coincide with swarming, I do not believe that they have any influence on the periodicity of swarming in the Oosterschelde.

Is there perhaps some influence of the phases of the moon, either in a direct way by regulating the dates of swarming; or in the way assumed by Orton, by regulating spawning and thereby causing a swarming maximum about 7–10 days after full-moon?

It is evident that swarming is not limited to the days of full and new moon or to the days, immediately following these phases, on which the spring-tides occur. This has never been observed in France either. Many swarming-maxima occur about 10 days after one of the extreme phases of the moon, e.g. 3–5 July 1937, 18–19 July 1937, early in July 1938, 21–23 July 1938, early in August 1938, 25–26 June 1939, 11 July 1939 and 12 August 1939, which points to the possibility of spawning-maxima during these extreme phases.

I would, however, emphasize the fact that, even if a maximum of spawning during the extreme phases of the moon (so during spring-tide) should occur, there is certainly no difference between the full-moon spring-tide and the new-moon spring-tide. So I can not bear out Orton (1926), who assumes a maximum of spawning during full moon only. I dare not yet decide if the periodicity of swarming in the Oosterschelde justifies the conclusion that most of the spawning occurs during the spring-tide. My data show, however, that spawning certainly is not limited to spring-tides, and in no case to the full-moon spring-tide. Moreover the diagrams show that spawning, which most probably occurs about 7–10 days before swarming, does not require high water-temperatures. We may state, on the contrary, that often a profuse spawning must have taken place during fairly low water-temperatures.

So summarizing our knowledge about the periodicity of swarming of *Ostrea edulis*, especially based on the observations in the Oosterschelde and on the graphs of the French investigations, I can state that:

1. Swarming is not a simple function of the actual water-temperature. The graph on the number of free-swimming larvae does not run strictly parallel to that on the water-temperature.

I would have it clearly understood, however, that this does not preclude all influence of the water-temperature on the periodicity of swarming. This influence is of a rather complex nature, however, as has been discussed in previous chapters. The mother-oysters do not await favourable weather before ejecting their larvae.

2. Slight variations in salinity apparently have no influence on swarming.

3. Swarming takes place during fine weather as well as during rainy weather. Strong winds do not prevent swarming.

4. Swarming is dependent on the periodicity of spawning. The duration of the period of incubation will vary to a certain degree with the water-temperature.

5. Spawning is not limited to the full moon spring-tide in Holland and France. There are indications, however, that a considerable part of the spawning tends to be concentrated at both of the springtides.

6. Spawning does not exclusively show its maxima during very high water-temperatures.

7. The periodicity of spawning is governed mainly by the periodicity of sex-change, which is in its turn dependent on temperature and probably on metabolic conditions.

It is not yet known if a chemical stimulation of some kind plays any part by causing a strictly simultaneous spawning of that percentage of oysters which is at that very moment ready to spawn. Temperature-conditions being propitious, we may expect 100 % or even more than 100 % of a population of *Ostrea edulis* to function as females every year, even in the countries around the North-Sea.

So far the only reliable method to record the rate of swarming is a frequent observation of the number of oysterlarvae in the water. I consider a frequent examination of fairly large samples of adult oysters in order to find the measure of spawning less suitable to ascertain the periodicity of swarming, for we shall always have to take into account that oysters at different levels

of the beds, so living under different conditions, and oysters of different age-classes, will probably show a different periodicity of spawning. By observing the number of larvae per unit of water this difficulty may be eluded.

No investigator in Europe has succeeded so far in deducing a reliable mathematical formula, exclusively built up of easily observable factors, such as water-temperature, for the purpose of forecasting swarming. Such a formula would render the time-consuming plankton-investigations superfluous.

In view of the complexity of the processes which precede swarming and the fitful weather-conditions on the Atlantic coasts of Europe, I do not expect that such a really reliable formula will soon be found.[1])

XII. THE EXTENT OF THE ANNUAL PRODUCTION OF LARVAE

The total amount of larvae which is produced annually on certain producing grounds is not always the same. There are several factors which govern the quantity of this production and it is interesting to find out to what degree these factors vary in the Oosterschelde. Some of them will be fairly constant, while others will show wide fluctuations. The latter will be mainly responsible for differences in the annual production of larvae.

These factors are:

a. The age at which oysters reach sexual maturity in the localities concerned.

b. The percentage of oysters participating in female reproduction every year.

c. The number of larvae produced by one oyster in the female reproducing phase.

d. The number of sexually mature oysters on the producing grounds and the proportion of the strength of the age-classes of which that number is composed.

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[1]) In a recent paper LOOSANOFF (1939) tells us about observations of a spawning by *Ostrea virginica* at water-temperatures below 20° C. Even the maximum bottomwater-temperatures in the entire region remained below this figure. These observations show that even the methods for predicting the time of spawning in *Ostrea virginica* are not yet infallible.

I believe that the age at which the oyster reaches sexual maturity will be fairly constant in one special locality, at least in localities like the Oosterschelde where the yearly differences in temperature are not very great. In waters where female reproduction takes place only at a fairly advanced age we need especially these older oysters to obtain an adequate production of larvae, while in other localities younger oysters will play an important part in reproduction, which is an advantage of paramount importance.

At what age does *Ostrea edulis* reproduce as a female?

Investigators in the South of France (Arcachon) stated that oysters of one year old, so in their second summer, are often found to carry larvae there (GERBE 1876, DANTAN 1913). DUPAIN (1932) is of opinion that the production of larvae by one-year old oysters, attached to the collectors ("piquetage") used in the estuary of the river Charente (farther north in France) is by no means negligible.

ORTON (1937 a, 1922 a) states that after exceptionally fine summers, like that of 1921, the female phase may be reached in 12 months in the English waters, but even there only exceptionally. This is confirmed by DODD, etc. (1937), who in 1936 detected some oysters containing larvae amongst a number of selected well-grown oysters, born in the fine summer of 1935.

SPÄRCK (1922, 1925) stated that in the Limfjord he never found an oyster carrying larvae in his second summer and that even the completion of egg-development in the course of the third summer is exceptional. As a rule the oysters in the Limfjord start female reproduction in their fourth summer, so when the animal is three years old. It will be clear that such a great difference between the ages at which the oyster reaches maturity in France and Denmark will govern the extent of the production of larvae to a high degree.

I examined many oysters in the Oosterschelde (Holland) and I observed that we may find a small percentage of one-year old oysters carrying larvae in their second summer (1937, 1938, 1939). It is not necessary for the preceding summer to be exceptionally fine, for even in 1937 (after the cold summer of 1936) I detected some one-year old oysters with larvae.

As it is, I consider the share in larvae-production of these one-year old oysters of so little importance, not only because of the small percentage participating, but also in view of the

small number of larvae in each of those oysters, that their influence on the total number of larvae produced is not likely to be of any importance in the Oosterschelde.

A discussion of the percentage of oysters participating in female reproduction is to be found in the sections on the phenomenon of sex-change.

There are many data available on the percentage of oysters that carry larvae simultaneously. The greater the frequency of sampling, the more valuable these data will be. From the latter the approximate percentage of oysters participating in female reproduction in one special year may be computed, especially when the frequency of sampling is fairly high and a reliable sampling of comparable oysters is carried out. However, I want to emphasize that such investigations will only yield data concerning the age-class from which samples are collected. Simultaneous sampling from several age-classes and in several places will be necessary if we want to form an adequate idea of the extent of the spawning-activities in the entire population.

SPÄRCK (1925) states that in the Limfjord about 14 % of the mature oysters may be expected to carry larvae simultaneously in the beginning of the season. This percentage decreases as the season advances.

HAGMEIER (1916) at List found about 10 to 15 %, that carried larvae simultaneously. ORTON (1926, 1928 a) states that in the English waters the maximum percentage of oysters carrying larvae simultaneously is usually about 25 % at the height of the season. Once he even recorded 33 %. ORTON computed that especially during fine summers more than 100 % of the adult oysters will function as females in the English waters.

HOPKINS (1937) investigated the closely related *Ostrea lurida* in the Puget Sound and found that a percentage of 15 to 20 % oysters carrying larvae simultaneously is common there, though peaks of 35 % and even 45 % may be found, especially in the beginning of the season.

Though I did not carry out frequent samplings of oysters in order to examine the percentage carrying in the Oosterschelde, I made some observations during the summer season on the percentage of incubating oysters, examining native oysters as

well as oysters imported from France. I carried out these
investigations in order to find out if subsequent swarming of
some importance was still to be expected; for instance, after
the failure in setting of the larvae of the first important maximum
of swarming in the beginning of July 1938. In that particular
case I forecast subsequent swarming, for both the native and
French oysters proved to be carrying larvae from 20 to 25 %
on July the 15th (1938).

Very interesting are the data obtained in 1939. As may be
seen from the diagram (fig. 10 a) heavy swarming occurred
on June 25/26 and 29/30, July 5, 11 and 24 and August 7,
11/12 and 22. Moderate swarming took place on June 19 and
August 4. Of less importance is the swarming on June 13.

In 1939 I ascertained three times in samples of 50 oysters
the percentage of oysters carrying larvae, each time in the
same three parcels of oysters. One parcel consisted of native
oysters of about 50 kg a 1000, the second parcel of newly-
imported French oysters of about 25 kg and the third parcel
of French oysters imported the year before (1938), weighing
about 45 kg.

I recorded:

	Natives			French 1939			French 1938		
	white	black	total	white	black	total	white	black	total
June 20	20%	22%	42%	14%	4%	18%	6%	8%	14%
July 12	4%	6%	10%	4%	0%	4%	4%	0%	4%
August 2	6%	4%	10%	16%	8%	24%	20%	0%	20%
			(62%)			(46%)			(38%)

Though the samples contain but 50 oysters I will yet assume
that these figures do not deviate too much from the real per-
centages. The examination of 12 July showed smaller percent-
ages, which is in accordance with the scanty swarming during
the days following on the 12th of July. The smaller percentage
of black-sick oysters on the 2nd of August is in accordance with
the moderate swarming on August 4, while the higher percentage
of white-sick oysters on that date accounts for the heavy swarming
on the 7th of August.

It will be clear that the incubation actually observed during
these investigations can, on a liberal calculation, only have

contributed to the swarming-maxima on June 25/26 and 29/30, August 4, 7 and perhaps also to that on August 11/12.

I actually observed that about 50 % of the oysters incubated. As these 50 % can only account for about half of the total swarming (probably for still less), I conclude that about 100 % of the total population must have been functioning as females in the Oosterschelde in 1939. It is my belief that the swarming towards the end of August, observed in 1938 and 1939, must be ascribed to oysters functioning as females for the second time during that season.

In any case my data on the periodicity of swarming, in combination with some data on the percentage of oysters carrying larvae indicate that at least 75 % of the adult oysters function as females annually, and sometimes probably more.

We already discussed that the frequency of sex-change will govern the percentage of oysters functioning as females during one season and that the frequency of sex-change is governed in its turn by the water-temperature and probably by the amount of nourishment. So cold summers may be expected to cause a decrease in the production of larvae by decreasing the percentage of oysters that function as females (e.g. 1936). Fine summers will produce the opposite effect.

There are many data on the number of larvae that may be found in one incubating oyster.

LEEUWENHOEK (1722) was the first to form an estimate, but he dared not mention the number, for he said they would not believe him anyhow! DAVAINE (1853) estimated about 1.125.000 larvae in one incubating adult and MOEBIUS (1883) found by weighing about 1.000.000 larvae in the mantle chamber of one adult. DANTAN (1913) carried out more detailed investigations at Arcachon in the South of France. He computed that one-year old oysters produce about 100.000 larvae, two-year old oysters about 250.000 larvae and older oysters about 750.000 larvae. As he found no difference between the numbers of "white" larvae and the number of "black" larvae, he concluded that no mortality or losses of any importance are likely to occur during incubation.

ORTON found 525.000 larvae in small three or four year old oysters from the river Blackwater and once 3.000.000 in a giant oyster.

All these data indicate that the usual estimate that marketable oysters (four or five years old) produce about 1.000.000 larvae during each female reproduction will be about correct. Stress must be laid on the fact that the proportion of the strength of the age-classes is of importance, as younger oysters produce far fewer larvae than older ones.

Ostrea lurida

Marketable Olympia oysters (*Ostrea lurida*), which are much smaller than *Ostrea edulis*, produce about 250.000 to 300.000 larvae, according to HOPKINS (1937).

Ostrea virginica

Non-incubating oysters produce far more eggs than incubating oysters, but the number of their larvae will have decreased considerably by the time they reach the stage at which the larvae of incubating oysters swarm, while in incubating oysters the number of swarming larvae is about equal to the number of fertilized eggs. NELSON (1921) estimates a spawning of 16.000.000 to 60.000.000 eggs in *Ostrea virginica*. The total annual production by one female of this species (which does not show a frequent sex-change!) is estimated by NEEDLER (1932 a) at about 500.000.000 eggs and by PRYTHERCH (1934 b) at about 100.000.000 to 500.000.000 eggs.

Ostrea gigas

GALTSOFF (1930 b) stated that the non-incubating *Ostrea gigas* will spawn 55.000.000 eggs at a time.

Though investigators are agreed on the average number of larvae produced by one female of *Ostrea edulis*, many investigators state more or less important annual fluctuations in this number, brought about by differences in external conditions.

SPÄRCK (1925) believes that not only the actual summer temperature governs the amount of eggs produced, but that temperature-conditions in the preceding autumn are also of influence, as egg-development often sets in towards the end of the preceding season.

HAGMEIER and SCHUBERT (1930) are also of opinion that the water-temperature during the preceding autumn affects re-

production. That the condition of the adult oysters will influence the number of eggs produced by these oysters is highly probable. Thus it is stated by GAARDER and SPÄRCK (1932) that feeding-conditions in the Norwegian pollen may show wide fluctuations. In summers with scanty sunlight there is but little algal growth in these pollen; bacteria and Peridinea will predominate then. This will result in a bad condition of the adult oysters; many of them will even die. Such summers are characterized by a decrease in the production of larvae, mainly owing to malnutrition of the adults.

ORTON (1937 a) is inclined to neglect the differences in the production of larvae: "There is no doubt that larvae occur in abundance in the water every year".

VOISIN states (1931): "Nous sommes donc encore obligés de constater toute l'obscurité qui entoure les différents facteurs d'influence qui régissent la ponte des huîtres. C'est pourquoi toute tentation pour prévoir à longue échéance l'époque et l'amplitude[1]) des pontes est, en état de nos connaissances, vouée à un échec".

Ostrea virginica

American investigators made more elaborate studies of the influence of external conditions on the amount of eggs produced by female oysters, especially by estimating and measuring the thickness of gonad-tissue shortly before spawning. NELSON (1928 a) states that a cold spring (e.g. 1926) results in a bad development of the gonads for lack of nourishment.

PRYTHERCH states (1929 a, 1934 b) that there is a marked correlation between the water-temperature from April to June and the production of larvae. The thickness of gonad-tissue varies from about 1,5 cm (e.g. 1925) to about 0,5 cm (e.g. 1926, 1927), resulting in important differences in the number of eggs produced in these years. During 1925 the water-temperature from April to August was above the normal. PRYTHERCH says that "it is likely that the fullness of gonad development is dependent on the amount of food consumed by the oyster'. PRYTHERCH (1929) points to the investigations by GALTSOFF (1928) on the correlation between the water-temperature and the volume of water sieved off by the gills

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[1]) Spacing is mine.

(*Ostrea virginica*). With the aid of these data obtained by laboratory-experiments and of data on the water-temperatures PRYTHERCH computes how many litres of water were sieved off by an average oyster during the months of April, May, June and July in the years 1922 to 1927 in Millford Harbor (Conn.). According to PRYTHERCH the normal averages are: April 25 litres, May 408 litres, June 816 litres, July 995 litres, total 2244 litres. A comparison of these years shows that the maximum was attained in 1922: 2551 litres and the minimum 1926: 2040 litres.

According to PRYTHERCH these relatively slight differences between the volumes of filtered water are responsible for the enormous annual differences in egg-production, the extent of which is directly deducible from the thickness of the gonad-tissues.

He does not mention the possibility that the amount of nourishment per litre may have varied, too, in those years. Unless we are given the amount of nourishment per litre, I do not believe that PRYTHERCH will ever convince us that only the slight differences in the litres of filtered water computed by him are really responsible for the differences in egg-production.

He estimates moreover the extent of egg-production during the years 1922–1927 from differences in the spatfall observed during these years, starting from the assumption that there is a direct correlation between egg-production and spatfall.

I disagree with PRYTHERCH when he states that the amount of eggs produced is the main factor governing spatfall, for the percentage of eggs becoming spat will vary according to external circumstances at the pelagic stage. Spatfall is no reliable standard for egg-production.

Ostrea edulis

What do we know about the variation in the production of larvae in the Oosterschelde? Though the water-temperatures since 1921 are known to me and I have also rough estimates of the spatfall during the years 1921–1935 at my disposal, I shall not try to compute from these data the influence of the water-temperature during the preceding autumns and springs on the extent of the production of larvae, as I am convinced that the spatfall is no reliable standard for the production of larvae.

However, these data do permit me to state that a relatively

poor condition of the oysters in the preceding autumn does not necessarily result in a poor spatfall in the next summer season.

Since 1935 plankton investigations have made it possible to form an adequate idea of the amount of larvae produced in in the Oosterschelde. These data can be computed from the diagrams by studying in particular fluctuations in the number of the youngest larvae. In comparing e.g. the diagrams for 1936, 1937, 1938 and 1939 we should bear in mind that in 1936 the samples were not collected exactly in the centre of larvae-production (the Yersche Bank) but at the station Strijen. A comparison of the data of these years at the station Kattendijke convincingly shows that the production of larvae in 1936 was much smaller than in subsequent years.

There has been a marked increase in larvae-production from 1936 to 1939.

Before considering the possible influence of temperature and feeding on the production of larvae, we shall try to estimate the number of adult oysters (oysters from their third summer) that were present on the oyster grounds in these successive years:

1936	about	14 500 000	adult	oysters
1937	,,	24 000 000	,,	,,
1938	,,	30 000 000	,,	,,
1939	,,	36 000 000	,,	,,

The increase in larvae-production appears to run parallel to the increase in the number of adult oysters. This does not mean, however, that differences in temperature and feeding-conditions may not also influence the production of larvae, but I believe the influence of the number of adult oysters to be very great.

When we compare the water-temperatures in the springs of these years (table 1), we find that it is impossible to show a marked correlation between the water-temperatures and the extent of the annual productions of larvae. The years when the larvae were very abundant (1938 and 1939) had cold springs, with water-temperatures below the normal. The year 1937 shows a spring with water-temperatures above the normal; still this year does not show an abundant production of larvae, not even when we take into account the above-mentioned increase

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in the number of adult oysters. The springs of 1935 and 1936 show approximately the same temperature-conditions, but in 1935 the number of larvae produced was higher than that in 1936. The production of larvae in 1936 was extremely poor, even when the number of adult oysters is taken into account, but temperature-conditions in that spring did not deviate much from those in the rich years 1938 and 1939. The years 1938 and 1939, with their abundant production of larvae, were preceded by autumns with rather favourable temperature-conditions, while 1936 and 1937 were preceded by autumns with lower water-temperatures.

This does not suffice, however, to convince me of the correctness of the assumptions of HAGMEIER and SPÄRCK, who state that the water-temperatures in the preceding autumn greatly influence the extent of the production of larvae.

I have no data about the influence of factors like quality and quantity of nourishment on the annual production of larvae in the Oosterschelde.

I agree with VOISIN that we have to await how many larvae will appear in the plankton each year. There is not yet a reliable formula to predict it. The larger the number of adult oysters, however, the greater the chance of an adequate production of larvae.

XIII. THE SIZE OF THE PELAGIC LARVAE

Ostrea edulis

MOEBIUS (1883) informs us that the larvae of *Ostrea edulis* measure from 0,15 to 0,18 mm (the greatest length of the shell parallel to the hinge) at the moment when they are set free. HAGMEIER (1916) organized his measured larvae (experiments on propagation in an enclosed oyster-pit) in three groups: newly-liberated larvae measuring 0,17 to 0,21 mm, grown-up larvae 0,22 to 0,26 mm and mature larvae 0,27 to 0,30 mm. HAGMEIER made several observations on larvae measuring about 0,21 mm, while still held in the mantle chamber of the adult. Moreover he obtained some indications that the size of the newly-liberated larvàe tends to decrease towards the end of the season of reproduction: "Es hat den Anschein als ob gegen Ende der Brutzeit die Grösse der frisch ausgestossenen Larven abgenommen hätte, doch reichen meine Messungen nicht aus um diese Tatsache einwandfrei festzustellen".

BOURY (1930) and VOISIN (1931) divide their larvae in larvae in the first stage and larvae in he second stage, the former being straight-hinged, the latter being provided with an umbo. Straight-hinge larvae measure from 165 μ to 228 μ, umbo-larvae from 200 μ to 295 μ; so both groups overlap. According to BOURY fixation takes place at a shell-length of about 270 μ, larvae larger than 270 μ being rare. ERDMANN (1934) informs us that the larvae of *Ostrea edulis* are set free normally at a shell-length of 0,16 to 0,18 mm. In the course of his experiments he found that a high water-temperature shortens the period of incubation, whilst decreasing the size of the freshly-liberated larvae, and conversely. A water-temperature of 23° C resulted in an incubation period of 6 to 8 days and in a shell-length of the liberated larvea of 0,16 to 0,17 mm. A water-temperature of 13 to 14° C resulted in an incubation period of 18 days and in a shell-length of the swarming larvae of 0,20 to 0,21 mm. So when HAGMEIER finds a decreasing shell-length of the swarming larvae towards the end of the season, this may be due to a higher water-temperature during incubation in the second part of the season. ORTON (1937 a) records that the ovarian egg measures 150 μ, the spherical segmenting embryo 130 μ, and the swarming larvae from 170 to 190 μ (l.c. p. 41); occasionally

TABLE III
THE SIZE OF NEWLY-LIBERATED OYSTERLARVAE

Date of swarming	Average temperature during incubation	Temperature at swarming	Distribution of the newly-liberated larvae over the size-classes			
			$15 \times 11\mu$	$16 \times 11\mu$	$17 \times 11\mu$	$18 \times 11\mu$
23 June 1938	16° C	18° C	1%	12%	41%	46%
28 June 1936	20° C	21° C	5%	13%	52%	30%
27 June 1938	17° C	18° C	1%	17%	46%	35%
13 June 1939	18° C	16,5°C	0%	15%	49%	36%
21 July 1938	17,5°C	18° C	6%	29%	50%	15%
19 June 1939	17° C	18° C	4%	28%	46%	22%
26 June 1939	18° C	18° C	4%	28%	41%	27%
11 July 1939	18,5°C	18,5°C	4%	39%	50%	7%
29 June 1939	18° C	17,5°C	5%	35%	46%	14%
9 July 1938	17° C	17° C	6%	35%	45%	14%
5 July 1939	18° C	18,5°C	5%	42%	43%	10%
7 Aug. 1939	19° C	19° C	14%	37%	42%	7%
8 Aug. 1938	20° C	23° C	9%	40%	35%	16%
4 Aug. 1939	19,5°C	19° C	8%	42%	41%	9%
24 July 1939	19° C	18,5°C	5%	45%	43%	7%
19 July 1937	20° C	21° C	10%	43%	34%	13%
18 Aug. 1937	20° C	19° C	9%	47%	35%	9%
22 Aug. 1939	19,5°C	20,5°C	11%	50%	29%	6%
3 July 1937	19° C	21° C	16%	52%	28%	4%
12 Aug. 1939	18,5°C	18° C	16%	54%	24%	6%
24 Aug. 1938	19° C	18° C	64%	30%	4%	2%

they may be found in the incubating mother-oyster with shells as long as 210 to 220 μ. (N.B. on page 118 l.c. ORTON declares that "the usual size at which they are set free is when the shell measures about 190 to 200 μ long by about 170 μ broad". This is not in accordance with his assertion on page 41!). According to ORTON mature larvae measure from 270 to 290 μ.

COLE (1939) states that at liberation his larvae measured from 0,16 to 0,20 mm, predominantly from 0,18 to 0,19 mm. Larvae from the same parent did not vary in size more than 0,015 mm. Recently set spat measured from 0,29 to 0,31 mm in his tank, the pigmentspot developed at a diameter of 0,27 to 0,28 mm. COLE mentions aberrations in both directions: large larvae of 0,33 to 0,35 mm, at a size of 0,33 mm often without any trace of a pigmentspot (tank-experiment 1937) and a case in which all the mature larvae remained under 0,285 mm and showed pigmentspots at 0,255 mm already (Helford River, Cornwall, 1938). COLE assumes that external conditions may be responsible for these large poorly differentiated larvae and these small completely differentiated larvae.

What about the size of the oysterlarvae in the Oosterschelde? From the diagrams we can derive data about the size of the larvae at liberation as well as at setting. So I sorted out a lot of marked swarming dates. A considerable swarming (on the Yersche Bank) causes a sharp rise in the number of larvae in consequence of the liberation of new larvae. Then the large majority of the larvae of the smaller size-classes may be consider-ed as newly-liberated larvae. So we can form an idea of the size of the larvae at swarming by comparing the number of larvae in each of the smaller size-classes on the dates of swarm-ing, although I admit that a slight interference by larvae that are already present before swarming sets in is unavoidable.

These data are arranged in order of a decreasing size of the newly-liberated larvae in table III. From this table we can deduce that in the majority of cases the size of the larvae at liberation varies between 0,175 mm and 0,185 mm. Larvae measuring from 0,165 to 0,175 mm are often recorded and are sometimes even abundant. I seldom or never found larvae of a size smaller than 0,165 mm in the plankton-samples. Sometimes a fairly consider-able part of the liberated larvae measure from 0,185 to 0,200 mm, but so far I have never recorded during swarming a

marked increase in the number of larvae of a size larger than
0,200 mm.

Further we may state that apparently temperature is not all-
powerful in this respect. Although in general high water-
temperatures during incubation coincide with a smaller size
of the liberated larvae, there are so many exceptions that I
cannot endorse unconditionally ERDMANN's assumption that
temperature during incubation regulates the size of the swarming
larvae. During the low water-temperatures in the first part of
July 1938 (16 to 17° C) I measured the larvae of many incubating
oysters, but I never recorded larvae larger than 0,200 mm in
the mantle chamber. I did not measure larvae incubated
below 16° C.

It is a striking fact that the liberations in June are all included
in the first part of the table, so among the young larvae of a
fairly large size, while the swarming in August, especially in
the second part of August, will always be found in the second
part of the table among the smaller larvae. Generally the water-
temperatures are somewhat higher in August, but this certainly
cannot always account for the smaller size of the August larvae
(e.g. 12 August 1939, incubated at 18,5° C). Moreover I recorded
the appearance of large larvae in the plankton during high
water-temperatures as early as 28 June 1936. In my opinion
the water-temperature during incubation probably has some
influence on the size at which the larvae are liberated, especially
when the differences are great, but moreover I am inclined to
assume that HAGMEIER was right when he supposed that the
size of the larvae tends to decrease near the end of the season. So
far I have recorded too few liberations in August during low
water-temperatures to be able to state with certainty that the
date of liberation is more important than the water-temperature
at the time of incubation, at least when the variations in temper-
atures are not very great.

Larvae provided with a pigmentspot (so-called mature larvae)
usually measure from 0,260 to 0,300 mm in the Oosterschelde.
Most of them are of a size from 0,275 to 0,285 mm. Mature
larvae somewhat smaller than 0,260 mm were only recorded
during the month of August. Larvae larger than 0,300 mm. occur
very rarely in the plankton of the Oosterschelde; the largest
planktonic larva I ever observed measured 0,315 mm. It is
my belief that the large larvae of COLE in one of his tanks are

certainly aberrant, but his larvae from Helford River are of a quite normal size, compared with the larvae in the Ooster-schelde.

A comparison of the number of oysterlarvae that may be found in 100 litres of water in the different centres of spat-production in Europe is hardly possible. The French investiga-tors do not know the volume of water they filter off with their plankton-nets. Though the German and Danish data also refer to net-towing, it is clear that larvae are far scantier in the Watten-meer and Limfjord than in the French waters. GAARDER and SPÄRCK (1932) counted 20 to 60 oyster larvae in 10 litres of poll-water (Norway), which corresponds with my data from the Oosterschelde. In the Oosterschelde a few hundreds of oyster-larvae per 100 litres may occur at the height of the season, even in places fairly remote from the swarming centre. During swarming quantities of 1000 larvae and more per 100 litres of water may be counted in the centre of larvae-production.

XIV. FOOD AND FEEDING OF THE LARVAE

ORTON's statement (1937 a) that the eggs of the European and kindred oysters are supplied with such a large store of reserve materials that the larvae will not need much actual food before settlement, must be taken with a grain of salt. The yolk-material of the eggs indeed renders active nourishment unnecessary for some time after the spawning of the eggs. Thus it has been stated by PRYTHERCH (1924) for *Ostrea virginica* and by FUJITA (1934) for *Ostrea gigas* that active nourishment only begins when a certain degree of larval development has been reached. Active nourishment in the larvae of incubatory species of oysters starts before swarming takes place, so during incuba-tion. It was DANTAN (1916) who noticed that larvae show a stomach content during incubation, which statement was con-firmed by ERDMANN's investigations (1934): "Ich konnte ein-wandfrei feststellen, dass sie während dieser Zeit schon aus dem die Mantelhöhle durchströmenden Kiemenwasser Nahrung zu sich nehmen".

YONGE (1926) has described in detail how the larvae feed. By feeding it carmin grains, east indian ink and iron-saccharate he was in a position to follow the entire process. From these

experiments we may conclude that the young larvae ingest anything of an adequate size. So the actual observations of the ingestion of a certain food material (HAGMEIER 1931: detritus, yeast-cells, starch grains, SPÄRCK 1927: Chlorella) does not prove that this kind of food can be digested by the larvae, at least not before the possibility of rearing the larvae with this food till fixation has been proved. KÄNDLER (1930) did not succeed in rearing the larvae by feeding them a pure culture of Chlorella, a non-motile alga.

The authors agree in stating that the larvae are unable to ingest food-particles larger than 8 to 10 μ. SPÄRCK (1927) observed that even *Nitzschia minutissima* is too large to be ingested.

It has appeared to be possible to rear oysterlarvae in vitro till settlement by supplying cultures of certain micro-organisms as food material (COLE 1936, 1938 b, 1939, ERDMANN 1933, HORI 1933, BRUCE and PARKE 1938, 1940). In co-ordination with his large-scale tank-breeding COLE (1939) carried out laboratory experiments, by which he proved that his original hypothesis (1936) that the larvae of *Ostrea edulis* require nude nannoplankton flagellates as food material is correct. So far he has only succeeded in obtaining setting in vitro by feeding the larvae cultures of such small nude flagellates. Non-motile green or blue algae of a suitable size proved to be useless to the larvae as food. BRUCE, KNIGHT and PARKE (1938, 1940) succeeded likewise in rearing the larvae till settlement by feeding cultures of several species of small flagellates (1–7 μ). Some species of flagellates proved to be more efficacious than others. In their most successful experiments the number of larvae reared from liberation to settlement exceeded 90%, in one case even 99% of the number introduced in the experimental vessel. The larvae are probably unable to digest the firm cell-walls of those non-motile algae, possibly because of the rapid passage of the food through the intestinal tract. COLE (1936) confirms the statement of SPÄRCK that non-motile algae of a suitable size are, however, readily ingested by the larvae. After settlement digestion of firm cell-walls becomes possible, so that non-motile algae can be used as food; meanwhile the quantity of food required increases considerably after metamorphosis.

COLE succeeded in stimulating the growth of these flagellates in his tanks and in preventing a predominance of undigestable

non-motile algae by the use of off-shore water and by an organic enrichment in the shape of a daily addition of a certain amount of ground crab to the water. In this way he obtained a rapid multiplication of small nanno-planktonic flagellates, so that adequate food-conditions are created for the larvae, which will then develop and settle in the tanks.

Hori (1933), however, succeeded in rearing the larvae of *Ostrea gigas* in vitro till settlement by feeding a culture of the non-motile alga *Chlorella pacifica*. It has not been made clear whether the larvae of this oyster are able to digest the cellwalls of such algae or whether it is the very thin cellwall of this species of alga (Uyeda 1927) which renders its digestion possible.

The Norwegian pollen have been closely examined (Gaarder 1932, Gaarder and Spärck 1932, Gaarder 1933), especially the Espevik-poll and the Saelø-poll. Owing to the exceptional hydrographic conditions in the pollen (discussed already in a previous section) the danger of a shortage of suitable food is not imaginary. Especially summers with scanty sunlight may be the cause of a very poor algal growth, causing a poor condition and even starvation of adult oysters.

The investigators inquired into the productivity of these pollen and effected an inorganic enrichment (nitrogen, phosphor and copper) to ameliorate the conditions for algal growth. They paid special attention to the occurrence of small non-motile algae, as they supposed that these might be a suitable food-material for oysterlarvae. This assumption was not based upon experiments in vitro. Gaarder and Spärck did not actually prove that non-motile algae can be digested by the larvae, although they readily ingest them. I will not absolutely deny that the high temperature of the poll-water ($\pm$ 25° C) may enable the oyster larvae to digest non-motile algae, but this remains still to be proved. Gaarder and Spärck cannot point to marked ameliorations in setting as the result of their experiments. Inorganic enrichment may cause rather dangerous changes in hydrographic conditions, as for instance an alarming rise of the pH by an increase in assimilation. Oysterlarvae die when the pH exceeds 9.0. Addition of copper counteracts algal growth and assimilation and thus the rise of the pH; additions of more than 0,040 mg Cu per litre are injurious to oysterlarvae, according to these investigators. Gaarder and Spärck added copper to the poll-water, as they thought it possible that it might contain

too little copper to render a good development of the larvae possible (PRYTHERCH 1931, 1934 a), although a real shortage of copper has never actually been demonstrated in the European oysterculture regions. The favourable effect of the addition of copper to the poll-water, as mentioned by GAARDER, was probably not apparent enough to allow of demonstration by numbers.

I did not carry out experiments to investigate this matter in the Oosterschelde. As general food conditions have appeared to be remarkably favourable to adult oysters here during the last few years, I am inclined to believe with ORTON (1937 a) that the absence of sufficient suitable food at the right time in the open sea is a possible, though perhaps not a probable factor Of course this problem is worth special investigation. I regret having to admit that we know but very little about the productivity of the Oosterschelde. We know practically nothing about the local variations in quantity and about the cycles of nitrogen, phosphor, manganese and other important elements, which in co-operation with other factors, like temperature and light, govern the productivity of the water.

XV. DISTRIBUTION OF OYSTERLARVAE DURING THEIR PELAGIC LIFE

Oysterlarvae are somewhat heavier than seawater. By ciliar action of the velum they are able to counteract the influence of gravitation. So by swimming or ceasing to swim they can to some extent control their vertical level in the water.

When we observe oysterlarvae in a small glass jar, we shall see that part of them are swimming actively and that another part is lying on the bottom, while again others may be found hanging quietly on the surface-film. When we stir the water vigorously, thus causing severe turbulences, all the larvae retract their velum and sink to the bottom.

It would be interesting to know whether oysterlarvae exhibit any rhythmical movements in the sea, whether they tend to accumulate near the surface at one time of the day or at a certain stage of the tide and to sink at other times.

We know beforehand that it is probably not necessary for larvae of *Ostrea edulis* to rest on the bottom or on the surface-film during their pelagic life. This is what we learn from the

experience in the Norwegian oyster-pools. The bottom of these pollen is rich in hydrogen sulphide (GAARDER and BJERKAN 1934), while oxygen is lacking. So the larvae cannot rest on the bottom there. The surface-layers are fresh or almost fresh, so that resting on the surface-film is also impossible. Nonetheless oysterlarvae develop till settlement in the pollen, from which we may conclude that resting on bottom or surface-film is most probably not necessary during pelagic life.

I shall discuss separately various factors which may affect vertical migrations, such as differences in temperature, salinity, light, current-velocity and the influence of wave-action on the vertical distribution of the oysterlarvae.

In another section special attention will be paid to the vertical distribution of mature oysterlarvae.

The influence of light on vertical distribution

PETERSEN (1908) told us about his observations in the Limfjord
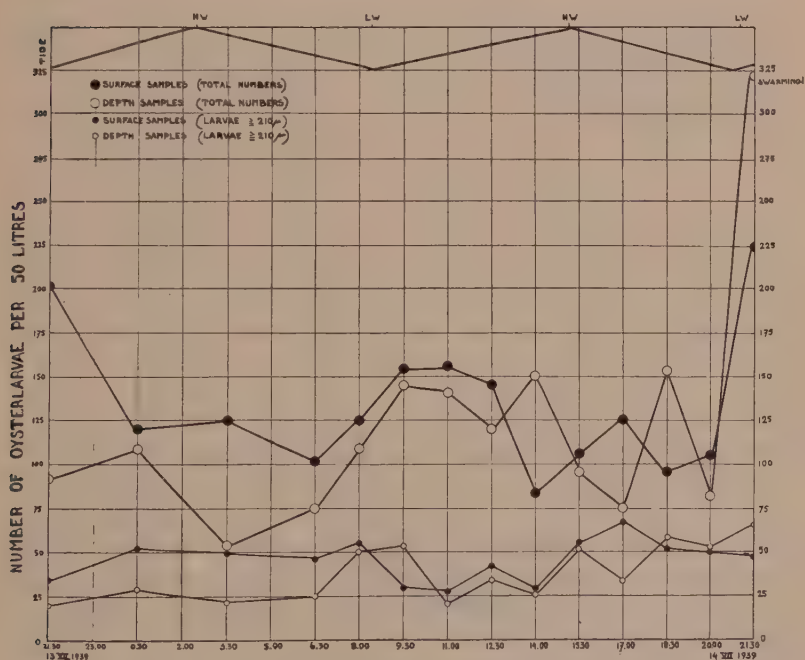
Fig. 11.   Number of oysterlarvae in the course of the tidal cycle. Station Yersche Bank.

"Both night and day, in bright, sunny and in dull weather, the spat was found on the surface, but constantly also in deeper water".

German investigators did not succeed in finding any appreciable influence of light on the movements of oysterlarvae in the course of their experiments in vitro (HAGMEIER 1932). Such observations in vitro (in a large container) had been made before by MAZZARELLI (1922), who did not observe any phototactical movements either: "O che il vaso sia tenuto nell' oscurità, o in piena luce, ovvero che sole un lato del vaso stesso sia colpito da raggi luminosi, restando nell' oscurità il resto, le larve dell' ostrica continuanno sempre ad aggirarsi in tutta la massa acquea, nella quale seguitano a distribuirsi in maniera affatto uniforme".

I did not come across other observations on the influence of light on the vertical movements of oysterlarvae. Nor did I
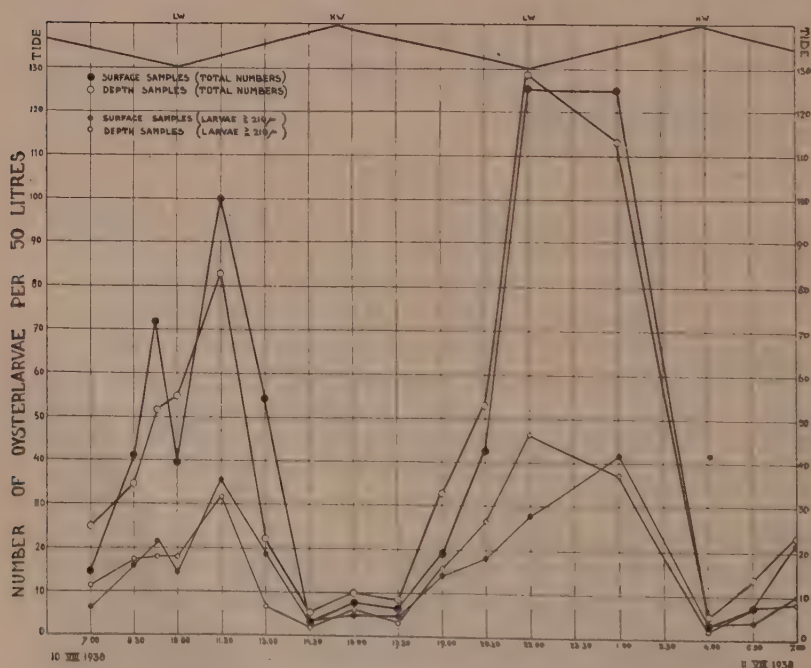
Fig. 12.   Number of oysterlarvae in the course of the tidal cycle.
Station Kattendijke.

find any comparisons of the number of oysterlarvae near the surface and in deeper layers during night and day.

In the summers of 1937, 1938 and 1939 I collected many special plankton-samples at different stages of the tides, sometimes continuing sampling for 24 hours in one station. Some of these series of samples were collected in the station Yersche Bank. During low water the depth is about 2,00 metres here, during high water about 5,00 metres. The samples were collected about 0,30 m below the surface and about 0,50 m above the bottom.

Other series of samples were collected at the station Kattendijke, where the depth is about 15 metres. Here I collected samples about 0,30 m below the surface and at a depth of about 5 metres below the surface. The bottom could not be approached here, as the length of the suction-hose did not allow of this.

The results of some of these investigations have been visualized graphically (fig. 11, 12). I shall often refer to these data.

In night-samples of 50 litres from the station Yersche Bank I counted e.g.:

Date	Hour	Surface	Bottom		
14 VII 1937	23.15	64	69	larvae	
19 VII 1937	24.00	106	156	,,	
22 VII 1937	24.00	50	44	,,	
22 VII 1938	24.00	112	124	,,	
23 VII 1938	1.30	113	60	,,	
23 VII 1938	4.30	155	175	,,	
15 VII 1939	0.30	118	109	,,	(fig. 11)
15 VII 1939	3.30	127	55	,,	(fig. 11)
		845	792		

There appears to be no appreciable difference in the number of larvae in the surface-layers and in the bottom-layers during the night.

The data on couples of night-samples collected at Kattendijke show a still closer similarity in the number of larvae in the surface-layers and at a depth of 5 metres (fig. 12). As will be discussed below, we shall always expect a somewhat irregular course of the number of larvae at the station Yersche Bank, owing to the proximity of liberating adults.

Some data on samples during d a y l i g h t are (also Yersche Bank, 50 litres):

Date	Hour	Number of larvae		
		Surface	Bottom	
15 VII 1937	10.00	43	60	
20 VII 1937	12.00	120	117	
23 VII 1937	12.00	50	72	
23 VII 1937	18.00	56	31	
22 VII 1938	7.30	298	189	
22 VII 1938	9.00	188	170	
22 VII 1938	10.30	75	158	
22 VII 1938	12.00	123	113	
22 VII 1938	15.00	149	102	
22 VII 1938	16.30	255	128	
22 VII 1938	17.15	192	227	
22 VII 1938	18.00	93	123	
15 VII 1939	8.00	126	112	(fig. 11)
15 VII 1939	9.30	158	144	(fig. 11)
15 VII 1939	11.30	153	138	(fig. 11)
15 VII 1939	12.30	147	120	(fig. 11)
15 VII 1939	14.00	82	150	(fig. 11)
15 VII 1939	15.30	109	93	(fig. 11)
15 VII 1939	17.00	123	75	(fig. 11)
15 VII 1939	18.30	97	148	(fig. 11)
		2637	2470	

So there is no considerable difference in the number of larvae near the bottom and in the surface layers during d a y l i g h t either. In this case, too, the couples of samples collected at Kattendijke (fig. 12) show a still closer resemblance as regards the number of larvae at the surface and at a depth of 5 metres.

So far my field observations have shown that the enormous difference in illumination between day and night does not seem to have any influence on the vertical distribution of the larvae of *Ostrea edulis*. Variations in illumination of less importance, caused e.g. by differences in cloudiness never appeared to have an appreciable influence on the vertical distribution of oysterlarvae in the Oosterschelde. This does not surprise me, since I failed to detect an influence of the difference in illumination between day and night.

All this in perfect agreement with the observations in vitro by MAZZARELLI and with those in Heligoland.

The influence of temperature on vertical distribution

There are only a few investigators who tell us something about the influence of temperature on the vertical distribution of oysterlarvae. MAZZARELLI (1922) noticed that his larvae in vitro sometimes rose to the surface in groups and thereupon sank to the bottom again in a way that strikingly resembled the movements of convection-streams. He assumed differences in temperature to be the cause of this phenomenon. HAGMEIER (1932) told us that such movements in vitro have also been observed in the Heligoland laboratory. The German investigators ascribed them likewise to convection-streams and to differences in temperature. When the temperature in the basin is perfectly equable, this phenomenon does not occur.

PERKINS (1931), inquiring into the vertical distribution of the larvae of *Ostrea virginica* in Barnegat Bay (U.S.A.), tells us that the part played by temperature in bringing about vertical migrations of the larvae is insignificant there. In Barnegat Bay the temperature of surface- and bottom-layers differed 1° or 2° C. There appeared to be no marked relation at any time between temperature and vertical distribution (field observations).

The tidal mixing of the water in the basin of the Oosterschelde is so thorough that marked differences in temperature between surface-layers and bottom-layers practically never occur. The differences in temperature between bottom-water and surface-water, read off by me whilst collecting the series of samples at the station Yersche Bank, always remained below 1° C and often were hardly perceptible. The temperatures at Kattendijke (surface and 5 metres below the surface) showed the same phenomenon. The insignificance of the differences is probably the reason why I cannot deduce from my data any apparent relation at any time of day or night between water-temperature and vertical distribution. This does not imply, however, that the larvae of *Ostrea edulis* are unable to react upon marked differences in temperature. The possibility remains that the vertical distribution is not the same during low water-temperatures (e.g. 16 to 17° C) as during high temperatures (e.g. 22° C). Although my special series of samples were collected during temperatures from 18 to 20° C, I do not believe in such a difference, however, for the diagrams on my daily samples do not provide the slightest indication of its occurrence.

Moreover it has been proved by MAZZARELLI (1922) that the larvae of *Ostrea edulis* show a uniform distribution in vitro at water-temperatures far below 16° C.

The influence of wind and wave-action on vertical distribution

BOURY (1930) compared the number of oysterlarvae in samples collected by net-towing along the surface and somewhat deeper. He collected some samples on a day with moderate wind and another number during slight air.

In spite of the fact that the towing of a plankton-net is not a reliable method of collecting quantitative samples, while moreover the number of samples collected was rather scanty and the differences in the number of larvae were not very considerable, BOURY concluded that wave-action expels the larvae from the surface layers: "Lorsque l'eau est calme les larves nagent donc surtout près de la surface; mais pour peu qu'il ait de vagues elles descendent vers les couches tranquilles". His compatriot BORDE (1931, 1932) tried to find out whether BOURY's assumption held good for the basin of Arcachon. He compared many samples collected by net-towing in different water-layers in various weatherconditions. BORDE was unable to confirm BOURY's data. He did not find relatively fewer larvae in the surface layers during rough sea than during smooth sea.

The data on my special series of samples allow me to inquire into this matter, too. I sorted out the couples of samples that were collected either during rough sea, caused by strong winds, or during smooth sea or slight rippling, during calm or slight air. The other samples were collected during intermediate degrees of wind and sea and are therefore less suitable for a comparison of the vertical distribution of oysterlarvae during smooth sea and rough sea.

These data show that in the Oosterschelde there is no appreciable difference between the vertical distribution of oysterlarvae during calm weather with a smooth sea and during rough weather. On an average the vertical distribution during smooth sea is similar to that during rough sea. During rough sea the oysterlarvae are not expelled from the surface-layers into the quieter deeper layers.

Station	Date of sampling.	Hour	Wind	Water	Number of larvae in 50 litres.	
					Surface	Bottom
Yersche Bank	10 VII 1937	18.30	strong	rough	92	47
(L.W. 2,00 m)	22 VII 1937	24.00	strong	fairly rough	50	44
(H.W. 5,00 m)	23 VII 1937	6.00	strong	rough	73	35
	23 VII 1937	12.00	strong	rough	50	72
	23 VII 1937	18.00	strong	rough	56	31
	23 VII 1938	6.00	fairly strong	rough	124	141
	15 VII 1939	3.30	fairly strong	rough	127	55
	15 VII 1939	15.30	fairly strong	rough	109	93
	15 VII 1939	17.00	fairly strong	rough	123	75
					804	593
	14 VII 1937	23.15	calm	smooth	64	69
	15 VII 1937	10.45	calm	smooth	43	60
	19 VII 1937	6.00	slight air	rippling	68	28
	19 VII 1937	24.00	calm	smooth	106	156
	22 VII 1938	7.30	slight air	rippling	289	189
	22 VII 1938	9.00	slight air	rippling	188	170
	22 VII 1938	10.30	slight air	rippling	75	158
	22 VII 1938	12.00	slight air	rippling	123	113
	22 VII 1938	15.00	slight air	rippling	149	102
	22 VII 1938	16.30	slight air	rippling	255	128
	22 VII 1938	17.15	slight air	rippling	197	227
	22 VII 1938	18.00	slight air	rippling	93	123
	22 VII 1938	21.00	slight air	rippling	150	93
	22 VII 1938	22.30	slight air	rippling	225	56
	22 VII 1938	24.00	slight air	rippling	112	124
					2137	1796
					Surface	5 metres
Kattendijke	10 VIII 1938	7.00	fairly strong	rough	14	25
(L.W. 12,00 m)	10 VIII 1938	8.30	fairly strong	rough	41	34
(H.W. 15,00 m)	10 VIII 1938	9.15	fairly strong	rough	73	52
					128	111
	6 VIII 1937	10.30	calm	smooth	27	40
	6 VIII 1937	16.30	calm	smooth	17	15
	10 VIII 1938	10.00	slight air	rippling	38	55
	10 VIII 1938	11.30	slight air	rippling	100	83
	10 VIII 1938	20.30	slight air	rippling	43	53
	10 VIII 1938	22.00	calm	smooth	127	130
	11 VIII 1938	1.00	calm	smooth	126	114
					478	490

The influence of salinity on vertical distribution

As far as I know only LEENHARDT (1924) mentions a possible influence of differences in salinity on the vertical distribution of the larvae of *Ostrea edulis*. LEENHARDT states that the larvae are expelled from the surface-layers during rainy weather. He does not, however, adduce facts to prove his statement.

My data do not point to any influence of rainy weather on the vertical distribution of oyster larvae[1]), but the possibility of such an influence in a very thin layer, immediately under the surface, remains, for I collected my surface-samples some 20 or 30 cm below the surface. In any case such an influence of rainy weather in such a thin layer will be negligible and it will have no appreciable effect on the distribution of all the larvae beneath this level.

Influence of salinity of another kind is discussed by American investigators for *Ostrea virginica*.

JULIUS NELSON (1916) noticed differences in vertical distribution at different stages of the tide. He believed that the larvae rise into the tide early in flow, and settle to the bottom before ebb begins and are thus able to migrate landwards and to avoid a rapid dispersion by the tidal streams. His son, THURLOW C. NELSON, assumed some years later (1921) that the youngest stages of the larvae do not show any marked difference in their vertical distribution; consequently they are carried seaward from the beds (surplus of ebb). Towards the end of the first week of larval life a distinct active reaction to the ebb and flow of the tide is evidenced: "within one hour after the tide has begun to fall the great majority of the oysterlarvae in the later stages have sunk to or upon the bottom. There they remain until the tide has begun to flow, when they swim upwards and are carried towards headwaters by the incoming tide. By repeating this performance with each ebb and flow the larvae are able by progressive stages to move upstream for considerable distances" (Delaware Bay).

NELSON carried out many investigations in Barnegat Bay

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[1]) The samples from the station Kattendijke collected on the 19th of August 1938 (fig. 2) were practically all procured in rainy weather.

(N.J.), which confirmed the occurrence of marked differences in the vertical distribution during different stages of the tide. Moreover he succeeded in finding the cause of this phenomenon. (1926, 1927, 1928 a, 1931).

Marked differences in salinity may occur there in the various water-layers. During flood-tide the heavier water of relatively high specific gravity creeps up along the bottom. The zone of transition may be very sharp (differences in sp. gr. from 1,010 to 1,018 for instance). In this zone, which lies immediately on the surface of the layer of dense bottom-water, the larvae of oysters and other animals may be found in comparatively enormous numbers. This discontinuity layer, where the upper more brackish water is in contact with the heavier more saline water, is called the halicline. NELSON states that it should not be assumed that the larvae "seek out" a zone of optimum salinity and congregate there. On the contrary, the effect of the salinity gradient appears to be one of purely relative salinity change. According to NELSON the congregation in the halicline is probably the result of a stimulus arising from rapid osmotic changes in passing from water of low salinity into that of higher salinity and vica versa. Once caught in the halicline, the larvae cannot easily escape from it: if they tend to sink into the heavier saline water, the rapid osmotic changes stimulate the larvae to swim upwards and when they swim into the more brackish layers, the larvae shrink back from the rapid osmotic changes in the other direction. As a rule this stratification disappears during ebb. Rough weather furthers a rapid mixing of the water. As the current-velocities may differ in the various waterlayers, the presence of the halicline and its influence on the vertical distribution of oyster-larvae may very well cause a passive migration in some horizontal direction.

NELSON often states that the larvae tend to accumulate near the bottom, when there is no halicline. His data do not prove this, however. When there is little difference in specific gravity between the water of the surface and the bottom, the larvae appear to be about uniformly distibuted (1928 a: fig. 2, diagram 2 and 3). The number of larvae near the bottom is then about equal to that near the surface. When there is a halicline, very few larvae will be found near the bottom in the layers of high salinity.

SEKI and TANAKA (1931) often noticed marked differences

in the number of larvae of *Ostrea denselamellosa* in samples from the surface and from deeper layers. Although their numbers of larvae (practically always less than 100 per 100 litres) are, strictly speaking, too small to base conclusions on, these differences may possibly be attributed to the differences in salinity that have been recorded by these authors, although differences in temperature, which occurred likewise, may also have exercised their influence.

I do not know whether the larvae of *Ostrea edulis* tend to react to marked stratifications of the water in the same way. The water in the Oosterschelde gets so thoroughly mixed by the tidal currents that any difference in salinity between the surface-layers and the bottom-water is hardly perceptible. The absence of a halicline in the Oosterschelde simplifies the study of the oysterlarvae there, as no interference on the part of the salinity is to be feared. It would be interesting to inquire into the behaviour of the larvae of *Ostrea edulis* in respect of salinity in a place where hydrographic conditions correspond with those in Barnegat Bay (N.J.).

The influence of the current-velocity on vertical distribution

The papers by American investigators mention two entirely different ways in which the current-velocity influences the vertical distribution of the larvae of *Ostrea virginica*.

In the first place there are the data on plankton-sampling in Milford Harbor (Conn.) and adjacent waters (PHYTHERCH 1929, GALTSOFF, PRYTHERCH and MAC MILLAN 1930). The investigators collected plankton samples by towing a plankton-net and by pumping up known quantities of water and filtering them through a plankton-net. Although profuse settlement occurs in the places where they sampled, they found but very few larvae in their samples. Many samples contained no larvae at all. They tried to find out the cause of this scarcity of larvae in the samples in these places, where settlement is abundant. The majority of a series of samples, collected during an entire tidal cycle, both from the surface and from the bottom-layers, proved to contain no larvae at all, while the number of larvae in other samples was very small. It should be remarked that the majority of these larvae were found to be in the later stages of development.

The largest number was counted in the samples collected at low slack water (e.g. 120 per 200 gallon). This led PRYTHERCH to assume that the larvae only swim roundabout slack water and rest on the bottom for the remainder of the tidal cycle. At high slack water the number was also very small, however, and the number of larvae at low slack water is minute in comparison with the numbers observed elsewhere on the east coast of North America.

PRYTHERCH assumes that the larvae drop to the bottom when the current-velocity exceeds a certain degree. Indeed he succeeded in finding some larvae in bottom material, collected at a moment of considerable current-velocity. Yet I believe that the number of larvae he found on the bottom is rather small in comparison with the heavy spatfall in that locality. PRYTHERCH tried to imitate the current-velocity in a large elliptical tank (1.000 gallon). The larvae in this container dropped to the bottom when the artificial current-velocities exceeded 0,3 to 0,5 foot per second. (8,5 to 14 cm per second). I want to remark, however, that an experiment in a tank like that does not reproduce natural conditions. The circular water movement in the tank introduces a centrifugal force, which complicates the situation. Moreover, water movements in containers are accompanied by more vigorous turbulences than watermovements in the open sea. Experiments in smaller jars have shown that the larvae soon drop to the bottom when vigorous turbulences occur.

The results of the investigations by other scientists are not in agreement with PRYTHERCH's assumption that the larvae of *Ostrea virginica* drop to the bottom when the current-velocity exceeds 0,5 foot per second. PERKINS (1931, 1932) for instance, found that the larvae are swimming actively during current-velocities that sometimes exceed 0,7 foot per second. In many places the larvae are abundant during all stages of the tide. It is still an unsolved problem why PRYTHERCH found so few larvae in the samples collected near Milford Harbor.

What do we know about this influence of the current-velocity on the larvae of *Ostrea edulis?* The fact that the vertical distribution of oysterlarvae in the Norwegian oyster-pollen is fairly uniform (GAARDER 1932) clearly shows that the absence of any current-velocity does have as a result that the larvae drop to the bottom-layers, as is sometimes supposed. HAGMEIER (1931, 1932) states that the larvae of *Ostrea edulis* do not tend to drop

to the bottom in quiet places in the open sea, as is often
assumed. Surface-samples collected in such quiet places do
not show far fewer larvae than samples collected from the
bottom-layers.

My series of samples collected in the Oosterschelde enable me
to inquire into this matter, as I collected several series of samples
during two entire tidal cycles at the station Yersche Bank. One
of them is visualized in fig. 11. The current-velocity in the
surface-layers exceeds 25 cm/sec about one hour after slack
water, about two hours after slack water the velocity is from
30 to 40 cm/sec. One hour before the next slack water the velocity
is again 25 cm/sec. So far I have never observed that the larvae
tend to drop to the bottom when the current velocity attains
these figures. It may be seen (fig. 11) that no marked peaks in
the number of larvae occur during slack water. The bottom
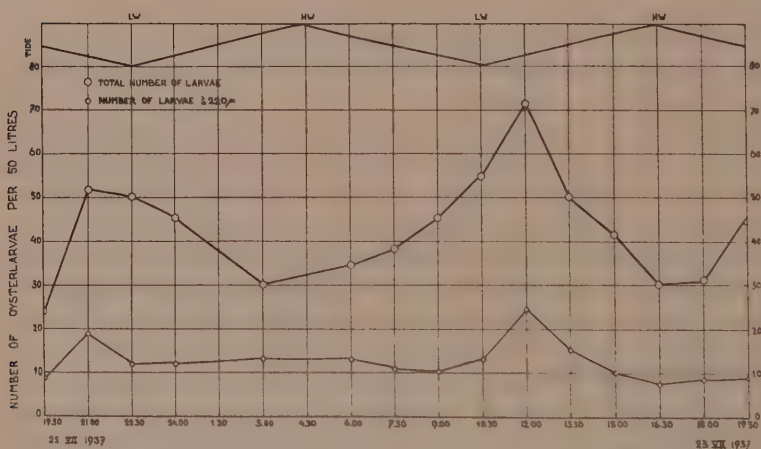
Fig. 13.   Number of oysterlarvae in the course of the tidal cycle.
Station 263.

samples were collected about 50 cm above the bottom, where
the current-velocity (measured by Rijkswaterstaat) is but a
trifle smaller than that at the surface. About 10 cm above the
bottom the current-velocity attains 20 to 25 cm/sec at the height
of the tide. The greatest number of larvae (300 per 50 litres)
in the series collected at this station on 22 July 1938, was counted
in the surface-sample, collected at half flow (at 7.30 h.),

when the velocity of the tidal current was about 40 cm/sec. The largest number I ever met with in the Oosterschelde (2000 per 50 litres) occurred in a sample collected likewise at half flow (at 18.00 h.) on the 14th of July 1937 at the same station. This enormous number must be attributed to the proximity of liberating adults.

In series of samples collected in station 263, somewhat farther westward (fig. 13), where current-velocities exceed 50 cm/sec for some time, I did not notice any dropping to the bottom either.

Even at Kattendijke (fig. 12) such a dropping does not occur, although here the current-velocity exceeds 50 cm/sec from one hour after slack water till one hour before the next slack water, often exceeding 100 cm/sec at the height of the tide. These samples do show marked peaks in the number of larvae during a certain stage of the tide (at low water), but it is clear that this has nothing to do with the current-velocity, for the peaks do *not* occur at *high* slack water. This phenomenon will be explained presently.

Although the current-velocities in the Oosterschelde far exceed 0,5 foot per second (the limit for active swimming, as assumed by PRYTHERCH), I never noticed that the larvae of *Ostrea edulis* tend to drop to the bottom during these strong tidal currents.

The larvae of *Ostrea denselamellosa* were collected by SEKI and TANAKA (1931) in large numbers during a current-velocity of 1,45 miles per hour. The surface-samples did not contain fewer larvae than the bottom-samples, so these larvae do not drop to the bottom either.

The other way in which the current-velocity influences the vertical distribution of oysterlarvae is discussed by PERKINS (1931, 1932). PERKINS worked in the same locality as NELSON: viz. Barnegat Bay N. J. He stated that often there is no apparent correlation between salinity and the distribution of larvae: "In fact the curve of vertical distribution was practically the same as when sharp stratification was evident". "When we see that concentrations of larvae occur at levels other than where salinity changes are great and that the distributional curve is practically the same whether a halicline is present or not, it is clear that here salinity is not playing a part. On the other hand the distributional curve does not follow the tidal velocity curve in its general

aspects". This he stated in his first paper (1931), after he had carried out some measurements of the tidal velocities at different depths, but without a simultaneous sampling of oysterlarvae at these various levels.

PERKINS does not believe that salinity is all-important in bringing about an orientation of bivalve larvae, but he assumes the current-velocity to be the cause of the inequal vertical distribution; according to him the increase of the number of larvae is commensurate with the increase in current-velocity.

PERKINS emphasizes that the larvae do not actively seek out the zone of greatest current-velocity, but that they are swept passively into the layer of increased current-velocity.

PERKINS explains the mechanism as follows: "It is a fact generally known to physicists that particles carried by a stream of liquid through a cylindrical tube become aggregated in the axis of the stream. This is due to friction against the walls of the tube which slows up the peripheral layer. Similarly in a shallow body of water such as Barnegat Bay there is friction against the bottom and at the surface water-air interface so that particles carried in the current tend to become aggregated in the axial stream and form, not a cone, as in the cylindrical tube, but a wedge. In this way the vertical distribution not only of oysterlarvae but of all other small larvae and lifeless particles may be accounted for".

The amount of suspended lifeless matter shows likewise a maximum in the zone of greatest current-velocity. In order to show that he was right in his suppositions, PERKINS collected in the next year some series of plankton-samples at different depths, at the same time measuring the current-velocity (fig. 14). It is his opinion that these data suffice to prove the correctness of his assumptions. In this second paper (1932) PERKINS admits that the larvae are found in the halicline, whenever current-velocities are small and salinity changes relatively great.

In the first place I want to make some remarks about his diagrams (fig. 14):

PLATE I. Oysterlarvae responding to salinity changes, which according to PERKINS only occurs when the current-velocity is negligible.

Fig. I. Larvae congregated in the halicline; the current-
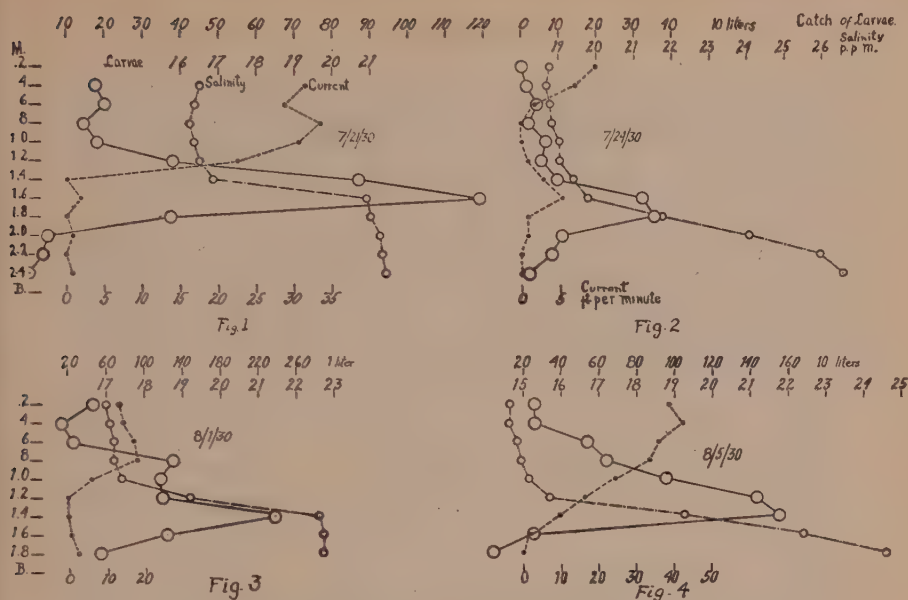
PLATE 1.   OYSTER LARVAE RESPONDING TO SALINITY CHANGES
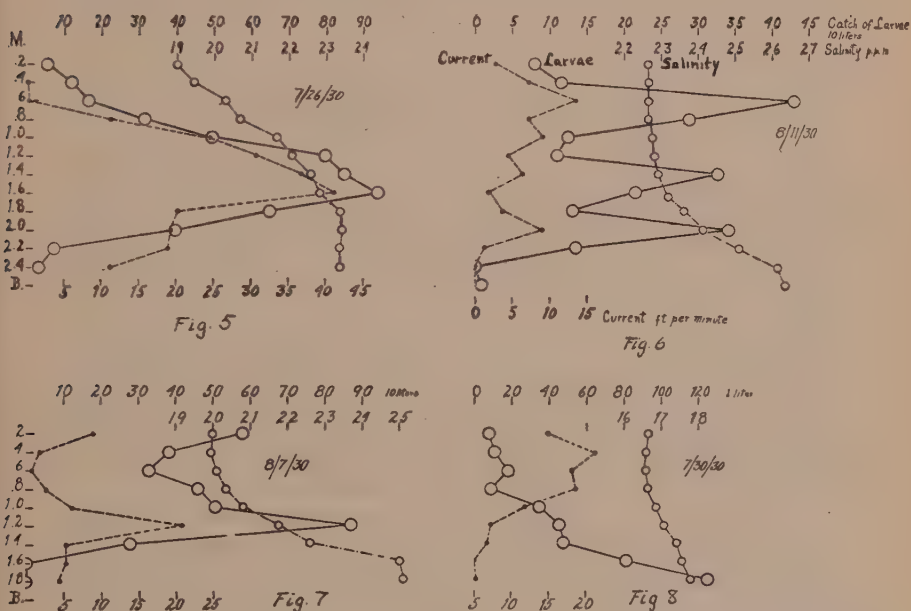
PLATE 2.   OYSTER LARVAE RESPONDING TO CHANGES IN CURRENT VELOCITY.   NO HALICLINE PRESENT

Fig. 14.   The influence of the current-velocity on the vertical distribution of oysterlarvae. After PERKINS, 1931. (Plate 1. Oysterlarvae responding to salinity changes; plate 2. Oysterlarvae responding to changes in current-velocity).

velocities in the upper layers, to 40 cm above the halicline, exceed 30 feet per minute! (The maximum current-velocity recorded in his diagrams does not attain 45 feet per minute). The current-velocity is certainly not negligible in this case.

Fig. 3. PERKINS ascribes a slight peak in the number of larvae above the maximum in the halicline to an increase of the current velocity in the layer in question. This current velocity remains below 20 feet per minute, so that action of the current velocity is more likely in the case visualized in fig. 1.

Fig. 4. The current-velocity exceeds 40 feet per minute and still the larvae respond to the halicline.

PLATE 2. Oysterlarvae responding to changes in current velocity.

Fig. 6. "Remarkable correlation between variations in current velocity and vertical distribution of oysterlarvae". The current velocity does not attain 15 feet per minute and in my opinion the number of larvae is not so remarkably commensurate with the current velocity.

Fig. 8. The current-velocity exceeds 20 feet per minute and might be expected to come into operation.

These figures show that the larvae are seldom abundant in layers with a salinity above 23 $°/_{oo}$. The only case of an abundance of larvae close to the bottom is represented by fig. 8, where the salinity remains below 18 $°/_{oo}$ to near the bottom. I believe that salinity plays a greater part than PERKINS is inclined to admit.

I do not believe that the forces which, according to PERKINS, are the cause of the difference in the vertical distribution, are strong enough to bring about a marked accumulation of the larvae in the layer of greatest current-velocity in the course of the few hours that the currents are rather considerable.

It is possible to compute the force exercised on a particle suspended in a medium of which the current-velocities in the different layers are not the same. TAYLOR (1928 a) gives a formula for that force. If we know the force, the size of the particle and the viscosity of the medium, it is possible to compute the velocity with which the particle tends to move towards the

layer of greatest current-velocity. This force is strong enough to bring about an accumulation of the larvae in a few hours, when the current-velocities are about 40 feet per minute.

In the case of oysterlarvae the particles are, however, not suspended, but they move along with the medium. The force exercised on a particle in this case is many times smaller than that in case of a suspended particle. Though TAYLOR gives the energy-function for this system water/particle (1928 b), it is very difficult to calculate how great the force really is, as some of the coefficients are difficult to compute.

Therefore I do not believe that the force in question is great enough to bring about the effect observed by PERKINS. PERKINS did not collect series of samples during an entire tidal cycle, so it is impossible to infer from his data in how far differences in the horizontal distribution of the larvae play a part in the vertical distribution. For if the larvae are transported by the currents from the centre of larvae-production to a station where the larvae are rather scarce and the current-velocities are different in the various water-layers, it is very well possible that we shall find a far greater number of larvae in the layer of greatest current velocity during a certain part of the tidal cycle.

The currents in the Oosterschelde are many times stronger than those observed by PERKINS. Consequently, if PERKINS' hypothesis is correct, an accumulation of the larvae in the layer of greatest current-velocity is bound to occur in the Oosterschelde. I never observed, however, a difference between the number of larvae near the surface (layer of greatest current-velocity) and near the bottom at the height of the tide and at slack water. I therefore conclude that the force exercised by a difference in current-velocity in the various water-layers is not strong enough in the Oosterschelde to bring about a marked difference in the vertical distribution of the larvae. As the currents were far slower in PERKINS' case, this force was most probably too small to have brought about the differences observed by him.

Movements in horizontal direction

PERKINS states (1931): "A knowledge of horizontal distribution is of extreme importance in determining the location of setting areas, because it has been known for many years that sets are heaviest in localities where the larvae had been most abundant." It is easy to understand that the presence of an adequate

number of mature larvae is the most important condition in procuring a spatfall of commercial magnitude. Several investigators have proved indeed that, generally speaking, the heaviest spatfalls are to be expected in those localities where larvae are most abundant (CHURCHILL and GUTSELL 1921, NELSON 1923 b: *Ostrea virginica*). It is impossible, however, to determine the horizontal distribution, unless vertical maxima are known.

Only PRYTHERCH (1929) assumes that the oysterlarvae are but to a negligible degree subject to the dispersing action of the tidal currents and that they wander only a few hundred yards from their place of origin. According to him the larvae have to rest on the bottom during by far the greater part of the tidal cycle to avoid dispersion. PRYTHERCH's assumption does not receive any support from other American investigators. Since my data concerning the larvae of *Ostrea edulis* in the Oosterschelde enabled me to prove that strong tidal currents do not cause the larvae to drop to the bottom, I do not think that it is necessary for me to take PRYTHERCH's views into account.

NELSON (1926, 1927, 1928 a, 1931) discusses a possible influence of vertical distribution (as brought about by marked differences in salinity) on horizontal distribution. When oysterlarvae tend to congregate in a special layer and the various layers show moreover different current-velocities, it is possible and even probable that horizontal distribution is affected by this state of affairs. It is doubtful, however, whether this influence is so efficacious as it was assumed to be by NELSON in a previous paper (1921), in which he supposed that the larvae migrate upstream. The influence of the salinity discussed above makes a movement upstream by progressive stages in principle possible, but the combination of the influence of salinity and that of different current-velocities may as well have a resultant in another or even in the oppostite direction.

There are not many data available on the horizontal distribution of the larvae of *Ostrea edulis*. German investigators (KÄNDLER 1928, HAGMEIER and SCHUBERT 1930) made some observations on the horizontal distribution of the larvae produced by the seed-oysters imported in the Wattenmeer. They found larvae in places fairly distant from the place of liberation and concluded that the larvae had been dispersed by the tidal streams. Many of them were washed away into the Northsea whence they but seldom returned.

BORDE (1932) stated that the larvae of *Ostrea edulis* are pro-
duced in a certain part of the basin of Arcachon, but that they
appear to be distributed over the entire basin after a couple
of days. HAVINGA (1932) and ORTON (1937 a) assume likewise
that the larvae are probably moved up and down in the tide
and that many larvae are carried away from the beds never
to return.

My special series of samples collected in various stations in
the Oosterschelde, where I often sampled for 24 hours at a
stretch, enable me to inquire into this matter.

I have already stated that the vertical differences in temper-
ature and salinity are always· negligible in the Oosterschelde,
so that complications in the vertical distribution of the larvae,
brought about by these factors, are not to be expected here.
Moreover the vertical distribution proved to be essentially the
same at slack water and at the height of the tide, when the current-
velocity exceeds 100 cm per second in many places. From this it
appears that the larvae of *Ostrea edulis* refrain from dropping to
the bottom when the current-velocity exceeds e.g. 100 cm per
second.

As differences in illumination and wave-action likewise ap-
peared to have no influence on the vertical distribution of the
larvae, we may conclude that in the Oosterschelde the larvae
are about uniformly distributed in a vertical sense at any time
of the day and the night, in all kinds of weatherconditions
and at all stages of the tide. As a result of this uniform vertical
distribution we may expect that the larvae are absolutely at
the mercy of the tidal currents. As to the direction and the force
of the tidal currents, I refer to the description in the chapter
on hydrographical conditions (fig. 1, 2).

The larvae are liberated in the basin of the Oosterschelde, so
that the very first larvae in the beginning of each new season
may be expected to appear at the station Yersche Bank. All
the swarming takes place in this region, which causes sharp peaks
in the number of larvae at this station. The tidal currents
disperse these larvae and distribute them over a large area. So
we shall find that the number of larvae at a station, situated at
some distance from the centre of swarming, tends to increase
some time after swarming; the greater the distance, the later
this increase is to be expected. It is self-evident that this dispersion
causes a decrease of the great number of young larvae counted

on the day of swarming in the centre Yersche Bank. The real
extent of swarming on one particular day can be deduced from
the degree with which the number of larvae increases at a more
distant station and from the number of larvae in the centre
after dispersion. The amplitude of the number of larvae during
swarming in the centre itself is a less reliable measure, as
it is affected by the degree of proximity of the liberating
adults. Thus the enormous number of larvae (2000 per 50 litres)
in a sample collected on the 14th of July 1937 at the station
Yersche Bank, for instance, did not point to a heavy swarming,
for the diagram (fig. 8) clearly shows that it must be attributed
to the immediate proximity of one (or more) liberating adult(s).

Very instructive is the number of larvae at the station Katten-
dijke in the course of a tidal cycle (fig. 12). As there are no
adult oysters in the neighbourhood, Kattendijke receives all its
larvae from the basin by the action of the tidal currents.

From the series of small charts (fig. 2) it may be seen that
the water which fills the basin at high-tide moves westwards
during ebb and reaches Kattendijke about two or three hours
before low slack water. Then for some time it moves still farther
westwards to return during flow. So the water at the station
Kattendijke during a few hours roundabout low slack water
consists of water that fills the basin of the Oosterschelde at
high water. About high tide the water at the station Kattendijke
consists of water derived from the "outlying district", situated
N.W. of this station. As the Channel of the Oosterschelde shows
a surplus of ebb, part of the low-tide water derived from the
basin will not return to the basin during the next flow, but will
mix with the high-tide water from the outlying district.

Two series of samples collected during 24 hours at this station
both yielded the same results. In fig. 12 the data from the sam-
pling from 10 to 11 August 1938 are visualized. They show a
marked correlation between the tidal cycle and the number
of larvae.

During low tide, when for some hours the water is made up
of the water that fills the basin during high tide, the number
of larvae is great, while the number of larvae counted in the
samples collected during high tide, when the water is derived
from the outlying district, is relatively small. The larvae
present during high tide at this station originate likewise from
the basin of the Oosterschelde, however; they are introduced

with the water of the successive surpluses of ebb which are mixed
with a great deal of practically "barren" water from the outlying
district. It will be clear that this will result in differences in
the composition of age-classes at high-water and at low-water. As
a matter of fact we do find that the high-water larvae are on
an average of a larger size than the low-water larvae. At this sta-
tion newly-liberated larvae are practically never met with during
high water. It may be seen in fig. 12 that at Kattendijke the
percentage of earlier larvae is much smaller during high-tide
than during low-tide. This series of samples provides at the
same time an instance of an almost negligible difference between
the samples collected near the surface and those collected at
a depth of 5 metres.
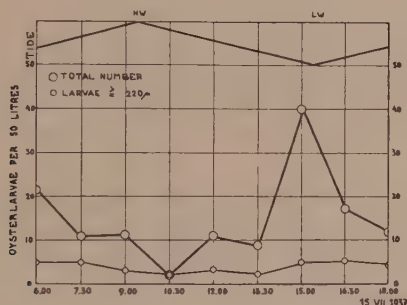
Fig. 15. Number of oysterlarvae in the
course of the tidal cycle. Station E.V.

When we collect a series of samples, still more distant from
the basin (e.g. station E.V. fig. 15), we shall expect a priori
(provided we do not cross the low-tide boundary of the basin-
water) a shorter period with ample larvae during low water
than at the station Kattendijke. Fig. 15, which represents such
a series, shows at the same time that the maximum number
of larvae during low-water (80 per 100 litres) remained consider-
able below the maximum at the station Kattendijke on that day
(about 150 per 100 litres). On 20 July 1936 we made a trip from
Bergen op Zoom to the outlying district (Zierikzee), on which
occasion we collected plankton-samples in many places. A sudden
drop in the number of oysterlarvae indicated that we had
crossed the boundary of the basin-water. This boundary appeared
to be situated between Wemeldinge and Kattendijke, about 4

hours after low-water. The next day, when we made the trip in the reversed direction, we traced the boundary somewhat N.W. of Kattendijke (near the station E.V.) about 4 hours after high water. This is perfectly in accordance with the data on the movements of the basin-water supplied by Rijkswaterstaat, visualized in the series of small charts (fig. 2). The point of the tongue of basin-water which intrudes in N.W. direction during ebb appears to be less rich in larvae than the rest of the tongue.

The point of the tongue is made up, generally speaking, of the water that enters the basin during the very last stage of the flow and which does not penetrate far into the basin. When the point of the tongue is less rich in larvae, we shall also expect fewer larvae in the western part of the basin during high-water. That this is really the case is shown by a series of samples taken in the western part of the basin (station 263, fig. 13). As here we are quite close to the centre of larvae-production the difference in the number of larvae between high water and low water is much slighter. As is to be expected, the average size of the larvae is largest during high tide, for during low tide we find more newly-liberated larvae.

I procured many series of samples during an entire tidal cycle and others during 24 hours at the station Yersche Bank, the same station where one of the daily samples is collected. I invariably noticed that the course of the number of larvae is not so neatly gradual as, for instance, at the station Kattendijke. I often met with peaks of greater or lesser importance and with differences between the number of larvae at the surface and the bottom.

A comparison of all these series of samples proved, however, that these peaks are not correlative with the tidal cycle and that the differences in vertical direction have nothing to do with variations in factors like illumination, temperature, salinity, wave-action and current-velocity. In fig. 11 such a series of samples is visualized. From this it may be seen that there is no regularity in the occurrence of the vertical differences in the distribution of the larvae.

In my opinion all these deviations from the "normal" course and from the "normal" vertical distribution, observed at this station, may be attributed to the proximity of liberating adults. This station is situated in the centre of the most important oyster-beds. The water that passes this place at all stages of the tidal cycle has flowed for some time over these oyster-beds.

Though swarming tends to be concentrated to a considerable degree, single liberating adults are practically never absent. The nearer the station of sampling is to the adults, the greater the chance that the larvae of one or more liberating adults will interfere with the normal regular course of the graph on the number of larvae.

I mentioned before that at this station I once counted 2000 larvae per 50 litres on a day on which the average number of larvae was about 100 per 50 litres. If my supposition that these irregularities at this station are mainly due to the proximity of liberating adults is right, we may expect these peaks to be caused by a sudden abundance of the youngest planktonic larvae, in other words, we may expect the graphs on the older larvae not to run parallel to the graph on the total number. This appears to be the case: when tracing a separate graph for each size-class I find that only the youngest larvae are guilty of the irregularities. Generally speaking we may state that the number of larvae at the station Yersche Bank does not show any marked tidal fluctuations.

Summarizing, I believe I may state that the larvae of *Ostrea edulis* are passively moved up and down in the tide.

The tidal currents disperse them and transport them. It has been shown that the data on these transports perfectly fit in with the data on the tidal movements supplied by Rijkswaterstaat. The water in the centre of basin of the Oosterschelde contains an approximately equal number of larvae throughout a tidal cycle.

Stations situated west of the basin are characterized by a smaller number of larvae at high-tide; the farther west the greater the difference between the high-water and the low-water number. The farther west the shorter the period with many larvae and the smaller the total number of larvae during the entire tidal cycle.

The larvae are apparently unable to counteract dispersion. This inability clearly shows the enormous advantage of sufficiently enclosed breeding-grounds. An adequate retention of the larvae is only to be expected in places where the tides perform a more or less perfectly oscillating movement, which enables the majority of larvae to return to the grounds after each tidal cycle.

In connection with the points discussed above I want to

9

make a few additional remarks about the reliability of my
daily samples (fig. 8, 9, 10). At the station Kattendijke the daily
samples are procured at low slack water. Roundabout low water
the number of larvae is great there. The moment of sampling
is seldom more than half an hour removed from the moment
of low slack water. Consequently errors caused by untimely
sampling are not to be expected. The depth of sampling is
always 5 metres here. As no variations in vertical distribution
occur here, errors owing to differences in illumination, salinity,
temperature, wave-action or tidal currents are not to be expected.
Consequently (and this may be deduced likewise from the dia-
grams) the abrupt transition from sampling at evening low water
to that at morning low water (indicated by "m" in the dia-
grams) has no influence whatever on the number of larvae
collected. Pump sampling at low slack water does not yield
unreliable results, for I never noticed fewer larvae in the samples
collected just at slack water (fig. 11, 12). Hence it follows that
the larvae are most probably not able to counteract percep-
tibly the suction of the pump by their own locomotive force.

At the station Yersche Bank samples are procured about half
an hour after low slack water and about two feet above the
bottom. Irregularities owing to the proximity of liberating adults
may occur here. The occurrence of swarming is soon noticed at
this station. The extent of each swarming can safely be deduced
from the number of larvae counted in the samples collected at
both stations on the day after swarming.

Weatherconditions, tidal currents, differences in salinity and
the abrupt transition to the morning-tides apparently have no
influence on the reliability of the sampling at this station either.

In order to explain the occurrence of fairly large numbers of
spat in places fairly distant from the spawning-grounds, several
investigators suggest a secondary accumulation of larvae. This
accumulation is not ascribed to differences in vertical distri-
bution by these authors.

They speak of eddies and of bights where the currents are
negligible. In places like these the larvae are supposed to accumu-
late by an unexplained mechanism (CHURCHILL and GUTSELL,
1921, NELSON, 1921, KÄNDLER, 1928, 1930). In places where
the same body of water maintains itself during an entire tidal
cycle the dispersion by the currents will be less effective. This
phenomenon is not observed in the Oosterschelde, however,

owing to the thorough tidal mixing and the considerable tidal range (3 to 4 metres).

This is not what these writers have in view, however; what they do think of is a secondary accumulation in such places after a previous dispersion: "Anderseits bewirkt die Boden-gestaltung im Verein mit Stromstauungen und Wirbelbildungen wiederum Anhäufungen von Austernlarven an einzelnen Stellen, die sich darum durch einen reichen Brutfall auszeichnen". (KÄNDLER, 1930). According to NELSON (1921) eddies act as a sort of "trap" for the larvae and he, too, mentions a heavy spatfall in such places.

They never state the real difference between the number of larvae in the bulk of the water and in such eddies. It is my opinion that the heavy spatfall in such places is what led to these suppositions. It will be discussed below that the propor-tion between the number of full-grown larvae and the intensity of setting is not the same at each station. Consequently there may be places, though remote from the centre of larvae-produc-tion, which may yet show an abundant spatfall. We need not assume a previous accumulation of the larvae to account for this phenomenon.

Unless we suppose that the larvae rest on the bottom during part of the tidal cycle or that circular currents cause centrifugal forces of some importance, I know of no other kind of mechanism capable of accumulating again the dispersed larvae. Since such a resting on the bottom does not occur in the Oosterschelde and as I have never met with such an accumulation, I do not believe that this phenomenon occurs here.

XVI. DURATION OF THE PELAGIC STAGE

Apart from the quality and quantity of the available nourish-ment, which factor has already been discussed in a previous chapter, there are some others which determine or are supposed to determine the general growth-conditions for the larvae and through this the duration of the pelagic stage. I shall respectively discuss the factors salinity and water-temperature.

The effect of salinity on the development of oyster-larvae

Many experiments in vitro have been carried out by several scientists in order to ascertain the optimum salinity for the develop-

ment of oysterlarvae. Larvae of non-incubatory species of oysters lend themselves admirably to this purpose, for artificial fertilization succeeds easily in these species. The following data have been obtained in this way:

Species	Salinity limits between which development is possible	Optimum salinity for early developmental stages	Salinity in the locality where these oysters grow wild	Author
Ostrea circumpicta Pilsby		28–38°/oo	23–35°/oo	AMEMIYA (1928*b*)
Ostrea denselamellosa Lischke	20–35°/oo	30–33°/oo	26–34°/oo	AMEMIYA (1921, 1928*b*)
Ostrea gigas Thunberg		15–25°/oo	10–32°/oo	AMEMIYA (1928*b*)
	8–35°/oo	18–27°/oo		AMEMIYA (1921)
		23–25°/oo		SENO, HORI and KUSAKABE (1926)
		20–22°/oo		FUJITA (1929)
Ostrea gigas var. *sikamea* Amemiya		15–25°/oo	8–30°/oo	AMEMIYA (1928*b*)
Ostrea rivularis Gould		15–25°/oo	10–30°/oo	AMEMIYA (1928*b*)
Ostrea spinosa Quoy.		30–38°/oo	25–33°/oo	AMEMIYA (1928*b*)
Ostrea virginica Gmelin.	15–39°/oo	25–29°/oo		AMEMIYA (1926)
	14,5–39°/oo	23°/oo		CLARK (1935)
Gryphaea angulata Lam.	21–43°/oo	28–35°/oo		AMEMIYA (1926)

Development often tends to become irregular and often shows a considerable retardation in cases when salinity is unfavourable.

It may be seen that there is in many cases a marked relation between the optimum salinity for development in vitro and the salinity in places where the species under consideration grow wild. During their larval life, too, littoral species are more eurihaline.

As regards *Ostrea virginica* some data on field-observations are available. NELSON (1921) states that oyster culture is possible between salinities of 10 to 31°/oo. HOPKINS (1931) made some observations in Galveston Bay (Texas), where considerable variations in salinity frequently occur. He assumed a correlation between increases in salinity and the intensity of setting. Although "quantitative methods of measuring the abundance of oyster-larvae in planktoncollections were not employed" and his meth-

ods of measuring the intensity of setting are not very reliable, he stated that "the results indicate that setting periods followed risings in salinity above approximately 20°/$_{oo}$, although it is certainly impossible to state an exact limit". As a matter of fact his data show that setting also occurred with salinities of 8 to 16°/$_{oo}$. A profuse spatfall was even observed at 12°/$_{oo}$ (l.c. p. 70 fig. 12).

As regards *Gryphaea angulata* it may be remarked that RANSON's (1938) observations are not in agreement with AMEMIYA's (1926) data. RANSON stated that no spatfall is to be expected in places where the salinity exceeds 24°/$_{oo}$. The natural beds of the Portuguese oysters are always situated in places with a salinity below this figure. In dry years the salinity in the French estuaries tends to increase to such a high degree that no spatfall of *Gryphaea angulata* takes place. BORDE and BORDE (1938) assume likewise that high salinities are unfavourable for the reproduction of the Portuguese oyster.

As artificial fertilization has been unsuccessful in *Ostrea edulis* so far and it is even impossible to rear young embryos taken from the maternal mantlechamber, the optimum salinity for development in vitro cannot be ascertained in the same way as in the non-incubatory oysters.

AMEMIYA (1926) tried to do so, but he stated that his results are not sufficient to base conclusions on.

GAARDER (1932, 1933) and GAARDER and BJERKAN (1934) assume that the larvae of *Ostrea edulis* require a salinity of 24°/$_{oo}$ or more for successful growth and presume that the optimum salinity will be about 30 to 35°/$_{oo}$. MAZZARELLI (1924) observed a normal larval development in the Lago Fusaro with salinities from 34 to 39,5°/$_{oo}$. The salinity during COLE's (1939) successful experiments in tanks varied from 30,5 to 32,5°/$_{oo}$. ORTON (1937a) assumes that the salinity of the water in the sea is probably rarely unsuitable for larval development.

Though the salinity in the Oosterschelde varies but slightly in the course of the summer, there may be some difference in salinity in different years.

The table on page 134 clearly shows that in the Oosterschelde there is no correlation between the intensity of the setting and the average salinity during the season of reproduction.

It is my opinion that variations in salinity between 25°/$_{oo}$ and

Year	Average Summer-salinity	Spatfall.
1922	29% ± 1	complete failure
1925	28% ± 1	good
1926	27% ± 1	profuse
1928	28% ± 1	profuse
1929	28% ± 1	very abundant
1931	28% ± 1	moderate
1934	29 to 30% ± 1	failure
1935	28% ± 1	good
1936	27% ± 1	complete failure
1937	26% ± 1	moderate
1938	28 to 29% ± 1	good
1939	27% ± 1	good

$35°/_{oo}$ probably have little or no influence on larval growth and development in *Ostrea edulis*. So I agree with VOISIN (1933) who states: "La salinité, si elle se maintient dans des limites normales, paraît ne jouer qu'un rôle assez effacé." Excellent spatfalls occurred in the Oosterschelde with salinities from 27 to $29°/_{oo}$, but other factors are probably responsible for the enormous annual variations in spatfall. I never detected any variation in the rate of development of the oysterlarvae in the Oosterschelde correlative with these slight annual differences in salinity.

The effect of water-temperature on the development of oysterlarvae

Just as in the case of salinity, it is easier to investigate the influence of the water-temperature on the development of larvae of non-incubatory oysters than of incubatory species. The study of the influences of temperature during early developmental stages in vitro is possible after artificial fertilization. The strictly simultaneous spawning in *Ostrea virginica* and *Ostrea gigas* facilitates the determination of the duration of pelagic life in the open sea.

Ostrea gigas

Fertilization takes place most vividly and the rate of development of the young larvae of *Ostrea gigas* proved to be most rapid at temperatures from 23 to 27° C (FUJITA 1929,

SENO, HORI and KUSAKABE 1926). ELSEY (1936) and ELSEY and
QUAYLE (1939) observed in Ladysmith Harbor that the duration
of the pelagic stage of this oyster is about 18 days at temperatures
of 21 to 22° C.

Ostrea virginica

Miss CLARK (1935) showed by experiments in vitro that
the rate at which early development takes place varies consider-
ably at different temperatures. The first swimming stage is
reached 25 hours after fertilization at 15° C and after 3 hours
at 30° C.

When the day on which the simultaneous spawning of *Ostrea
virginica* occurs is known, it is possible to ascertain the length
of the pelagic stage by determining the moment at which the
first spatfall takes place.

JULIUS NELSON (1908) presumed that the pelagic stage lasted
from one week at 24 to 27° C to three weeks at 21 to 24° C.
CHURCHILL and GUTSELL (1921) mentioned a free swimming
stage of 12 to 14 days at 21° C. NELSON (1923 a) stated 15 days
of pelagic life in Barnegat Bay, while the temperature varied
from 21 to 24° C; later on (1928 a) he stated 13 days at tempera-
tures of 23 to 25° C and 17 days when the water was 20° C.
According to NEEDLER (1932 a) the free-swimming stage often
lasts three weeks in the colder water of Canada. PERKINS (1932)
states that low water-temperatures are often the cause of a
protracted free-swimming period. When high water-tempera-
tures are recorded (25° C and more), fixation will be observed
from the twelfth day after spawning. MEDCOF (1939) carried
out investigations in Canada (Bideford River). He stated that
the duration of the pelagic stage there is about 30 days at 19° C,
about 26 days at 20° C and about 24 days at 21° C, which
figures are considerably higher than those of CHURCHILL and
NELSON. It is doubtful, however, whether his dates are absolutely
reliable; MEDCOF admits that his constructions are partly con-
jectural.

The determination of the length of the free-swimming periods
presents more difficulties in incubatory species of oysters, because
these oysters do not show a strictly simultaneous swarming. It
is only possible to speak of maxima of swarming, for during the
season of reproduction hardly a day passes with no swarming
at all. Swarming usually initiates with the appearance of a

small number of larvae in the plankton, practically none of which may reach the fixation stage, because of their small number. The measuring of the duration of the free-swimming period by ascertaining the period of time elapsing between the appearance of the first larvae in the plankton and the observation of the first spatfall must therefore be regarded as an altogether unreliable method. It is necessary to ascertain the interval between a maximum of swarming and a maximum of setting in order to know the duration of the pelagic stage in the field. For this a very frequent examination of larvae and setting is required. Often the spatfall does not show clearly marked maxima, which makes the determination of the length of the pelagic stage practically impossible in such cases.

Ostrea lurida

STAFFORD (1914) estimated the length of the free-swimming period in the Olympia oyster at about one month.

It was GALTSOFF's (1929) opinion that the length of this period is about 14 days. COE (1932 c) even presumed that the free-swimming stage is so short in *Ostrea lurida* that the chances of a dispersion of larvae by the currents are limited.

Neither of these authors had many reliable data at their disposal.

It was HORI (1933) who reared these larvae in vitro till fixation in 22 days at 20° C by feeding them ground sea-lettuce.

HOPKINS' (1937) investigations enabled him to establish the dates of spawning and thus to ascertain the maxima of swarming in Olympia oysters. Quantitative investigations on the abundance of larvae in the water were not made, however, so it was impossible for him to follow the larval age-groups. HOPKINS examined the spatfall, too, and it is possible to read from his diagrams (e.g. fig. 40) that the period which elapses between spawning and setting exceeds one month (17 to 18°C). So HOPKINS' conclusion that ,,the free-swimming period appears to be 30 to 40 or more days, depending largely perhaps on water-temperatures, so that the total larval life is at least 40 days'' will not be far from the truth. This shows that STAFFORD's first estimate was about right.

Ostrea edulis

PETERSEN (1908) observed that the larvae of *Ostrea edulis* swim actively in water of 13° C. He detected the presence of larvae in the plankton at 15° C. HAGMEIER's (1916) data on tank-breeding enabled him to state that the free-swimming period lasts from 10 to 14 days at 18 to 21° C. MAZZARELLI (1922) succeeded in rearing the larvae of *Ostrea edulis* in vitro till settlement. The water-temperature in his containers was not constant during his experiments. He stated that the duration of the free-swimming period is about 16 to 17 days at a water-temperature from 15 to 16° C. The water-temperature increased during his experiments, so that the temperature in the first part of the free-swimming period during which, in my opinion, the larvae are most sensitive, must have been fairly low (14 to 15° C.)

MAZZARELLI found viable larvae in the water of the Lago Fusaro at any temperature between 13° C and 30° C. He assumes that the larvae of *Ostrea edulis* can stand any variation in temperature between these figures.

BOURY (1928) concluded that he could not state a limit below which larval development and fixation are not possible.

KÄNDLER (1930) stated that the larvae require a temperature of about 20° C to grow and settle: "Erst etwa von 18° C ab gestatten sich die Entwicklungsbedingungen günstig und die Wassertemperaturen müssen sich eine Zeitlang um 20° C und darüber halten, damit die Austernbrut heranwächst und sich festsetzt."

There are more statements to this effect, for instance that by GAARDER (1933) who says that the larvae require 20° C for normal development and that by LAMBERT (1935) who tells us that no fixation takes place below 18° C.

I want to emphasize that it is wrong to argue on the assumption that no development and no fixation are possible below these temperatures, if this assumption is based on the fact that one does not succeed in finding spat after swarming during a cold spell. When the larvae are not very abundant and the pelagic stage is protracted owing to low water-temperatures, the number of larvae that survives till fixation may be so small that it is extremely difficult to find some spat. The experiments by MAZZARELLI prove that larval development and spatfall are certainly possible at fairly low water-temperatures.

CHAILLÉ(1938) expresses himself more cautiously when he states: "Dans nos eaux on peut dire qu'au dessous de 18 degrés la récolte est pratiquement nulle; les huîtres pondent, les larves sont émises, mais elles périssent avant d'avoir atteint leur stade de fixation."

ERDMANN (1934) observed that fixation in vitro is still possible after very long periods of pelagic life (e.g. 50 days). COLE's experiments in tanks (1936, 1939) yielded some further data. He observed fixation 9 to 10 days after swarming and concluded that the pelagic stage was much shorter than had been assumed by HAGMEIER and MAZZARELLI. COLE forgot to mention, however, that the water-temperature in his tanks was 21 to 22° C, which temperature is much higher than that in the experiments of the above-mentioned authors. From the data in his second publication it may be seen that the length of the free-swimming period in his tanks was about 10 to 11 days at temperatures from about 19 to 20° C.

Summarizing, we find that the following figures have been stated:

temperature	Duration of pelagic stage	Author
15–16° C	16–17 days	MAZZARELLI (1922)
18–21° C	10–14 days	HAGMEIER (1916)
19–20° C	10–11 days	COLE (1936)
21–22° C	9–10 days	COLE (1939)

The length of the pelagic stage in the Oosterschelde can be approximated by comparing in the diagrams the maxima of swarming with the maxima if setting.

It should be borne in mind that these comparisons are somewhat arbitrary, for the setting was not ascertained daily, but in periods of three days, as will be discussed below. The centre of such a period may be assumed to represent about the right place of the maximum of setting. Sometimes no marked maxima of setting occur. Maxima of swarming are also often not clearly marked and secondary waves of swarming frequently interfere. (See table on page 139.)

These data do not differ essentially from those of other investigators collected in the above table.

Temperature during the first 5 days	Temperature during the next days	Duration of pelagic stage	Dates	
			swarming	setting
16° C	17° C	14 days	8–9 VII 1938	22 VII 1938
17° C	16° C	13 days	27 VI 1938	10 VII 1938
17° C	17° C	12 days	23 VI 1938	4 VII 1938
19° C	18–19° C	12 days	17–18 VII 1939	29 VII 1939
20–21° C	18–19° C	9–10 days	18–19 VII 1937	29 VII 1937
21° C	21° C	7 days	21 VI 1936	28 VI 1936
22,5° C	22° C	7 days	11–12 VII 1935	18–19 VII 1935
22° C	23° C	6 days	2–3 VIII 1938	8–9 VIII 1938

I want to conclude that the length of the pelagic stage is largely dependent on the water-temperature and that COLE underestimates this influence when he suggests that the lengthening of the free-swimming period by water-temperatures as low as 15 to 17° C is not likely to exceed one day or at most two.

Though we know the length of the free-swimming period and also the measure in which the size of the oysterlarvae increases during this period, we are not justified in computing the daily growth-rate by a simple division of these two figures. It is very well possible that the daily growth-rate varies considerably in the course of the pelagic period.

As far as I know only NELSON (1923 b) and MEDCOF (1939) published some data about the daily growth-rate of the larvae of *Ostrea virginica*. NELSON measured regularly a number of larvae from eggs spawned simultaneously on one particular day in Barnegat Bay. The growth-curve proved to be of a sigmoidal shape; so the growth-rate appears to increase during the first part of the pelagic stage and to decrease during the second part. I doubt, however, whether the last part of the curve is quite reliable, for fixation, unlike spawning, is not quite simultaneous in *Ostrea virginica*. NELSON measured a certain number of larvae (10) and not the larvae from a certain volume of water; consequently we do not know how many of the original larvae had settled already. However, setting will no doubt influence the average size of the larvae during the very last part of the pelagic stage. In case we observe a "decrease in growth-rate" shortly before setting, by measuring a certain number of larvae,

there is a possibility that such a decrease must be attributed to fixation of the most precocious larvae. For these precocious larvae disappear from the plankton by attachment, so that only the late-comers get measured.

MEDCOF (1939) constructed his growth-curves with the aid of frequency polygons for larval measurement, plotted on a percentage basis. He did not sample daily and secondary waves of spawning interfered. Though his constructions are partly conjectural, we may yet conclude from them that the growth-rate increases in the course of the pelagic period.

A close study of my diagrams (fig. 8, 9, 10) may yield some data on the daily growth-rate of the larvae of *Ostrea edulis* during their pelagic life. It should be borne in mind that the division in size-classes in the diagrams does not imply a division in age-classes! There is an apparent difference in the proportions between the numbers of larvae in the different classes of the smaller sizes (165 μ to 210 μ) and between the classes of the larger sizes (210 μ to 300 μ). The decrease in the number of larvae in the course of the pelagic stage appears to be far most considerable in the groups of the smaller sizes. This phenomenon is not due to the dispersal of newly-liberated larvae, for the diagrams for Kattendijke show the same facts. Such a phenomenon may be attributed to the operation of two factors, a differential death-rate and a differential growth-rate. In this particular case I mean by the word "death" a disappearance of the larvae from the plankton in the basin, which may be caused by real death or by dispersion of the larvae to other bodies of water. The larvae of all size-classes appeared to be equally subject to the dispersing action of the currents (see vertical distribution).

A great many plankton-eating animals are the cause of the untimely death of countless oysterlarvae and although it is possible that some of those animals prefer to feed on the youngest stages or are not able to ingest the larger larvae, I do not believe that such a preference can be the only cause of the considerable difference in the proportion of the numbers of larvae in the various size-classes. It is my opinion that this difference in casualities points to the occurrence of a differential growth-rate. If we assume that the daily "death"-rate through dispersion and devouring is about the same in the course of pelagic life, but that the growth-rate of the larvae increases (so that a very rapid growth occurs after the size of 210 μ has been attained),

we shall expect a difference in the proportions of the larval groups as deducible from the diagrams.

It is my belief that my data point to the probability that the growth-rate increases considerably in the course of the pelagic life of *Ostrea edulis*, so that growing from 210 μ to 300 μ requires far less time than growing from 170–180 μ to 210 μ. Such an increasing growth-rate is in agreement with the result of the American investigations on *Ostrea virginica*; my diagrams do not point to any decrease of the growth-rate near the end of the pelagic stage, however.

XVII. DESTRUCTIVE AGENCIES

If all the larvae which are produced in one single year in the basin in the Oosterschelde should reach the adult stage, the basin would be filled up with them to above the high-water mark. A very high percentage of the larvae disappears from the plankton before the setting stage is reached. Only part of the mature larvae succeed in finding a suitable cultch to attach themselves and only a small percentage of the newly-settled spat will reach the adult stage.

The destructive agencies which cause the disappearance of so many oysterlarvae from the plankton may be divided in abiotic and biotic ones.

Abiotic destructive agencies
Tidal currents

All things considered, the tidal currents cannot be classed among the destructive agencies in a narrower sense, for they do not cause an untimely death of planktonic oysterlarvae. Nevertheless the disappearance of a considerably part of the oysterlarvae from the plankton above the oyster-grounds is due to the action of the tidal currents. Many of the larvae which are swept away during ebb do not return during the next flow. Hydrographic conditions determine what percentage of the larvae shall be carried away from the oyster-grounds by the tidal currents. It has been discussed in a previous chapter that the water renewal during each tidal cycle in the basin of the Oosterschelde is but slight. The larvae which do not return to the basin are still alive when they arrive in other bodies of water and probably growth-conditions there do not differ much from those in the basin. Those larvae are dispersed to such a

degree, however, that the number of larvae per 100 litres of water is so small in the outlying district, that the planting of cultch material there would not be justified from a commercial point of view. As a great part of the subsoil in the outlying district is absolutely unsuitable for the fixation of oysterlarvae and natural cultch material is very scarce, by far the greater part of the oyster-larvae which are carried away by the tidal currents to the out-lying district will not be able to find a suitable place to settle. Consequently those larvae have not only disappeared from the basin, but they should be considered as lost for the greater part.

In the section "Hydrographical conditions" it has been stated that about 4% of the water of the basin of the Oosterschelde disappears during each tidal cycle, carrying its plankton along with it. After 14 days about 35% of the original water is still present in the basin. The toll levied each tide of the oysterlarvae by the tidal currents is consequently not very great in the Ooster-schelde. When the pelagic stage is protracted by unfavourable temperature-conditions, the percentage that remains in the basin will be smaller than that which remains when the pelagic stage is short. We are, however, not justified in deducing from the foregoing that, in case of a pelagic period of 14 days, about 65% of the larvae initially present in the basin disappear from the plankton by the action of the tital currents, for there are still other factors causing loss of larvae. Consequently the 4% loss by the tidal water-renewal forms only a part of the total tidal loss, so that the losses during the next tide must be substract-ed from a figure smaller than 96% of the original number of larvae. Consequently the total loss caused by the tidal currents in the cause of the pelagic stage is not $(100 - 0{,}963^n \times 100)\%$ of the original number, but far less. How much it is in reality will be discussed presently.

Water-temperature

CHURCHILL (1920) and NELSON (1920, 1921) assume that the larvae of *Ostrea virginica* are extremely sensitive to sudden decreases in the temperature of the water. "A drop within 24 hours of from 3° to 5° may be followed almost immediately by the disappearance of a large part of the larvae from the water" (NELSON 1921).

PRYTHERCH (1929) and GALTSOFF, PRYTHERCH and McMILLAN (1930), on the contrary, stated that their studies had shown

that oysterlarvae can stand sudden changes in temperature. PRYTHERCH does not even take into account the effect of biological conditions during the pelagic stage on the percentage of larvae that reaches maturity. He assumes that especially the number of eggs produced determines whether the crop of spat will be sufficient or not. I believe that this view should be attributed to the circumstance that PRYTHERCH could not follow the fate of the larval herds, because of the scarcity of larvae in his samples.

Miss CLARCK (1935) observed that the larvae of *Ostrea virginica* (in vitro) can stand considerable differences in temperature. Decreases in temperature do not so soon cause a considerable mortality.

MAZZARELLI (1924) stated that the larvae of *Ostrea edulis* can stand temperatures from 12° to 30° C at least. Sudden changes in temperature do not cause a heavy mortality. The larvae swim actively at 13° C. SPÄRCK (1927) transported oysterlarvae in thermoflasks with ice (0° C). The changes from 18° to 0° and later on from 0° to 18° did not do any harm to the larvae of *Ostrea edulis*. Later on (1929) he proved that the larvae can stand a temperature of 0° for 24 to 48 hours, even of –2° C for a short time and of about 5° C for several weeks. So COLE (1939) is right when he states that "once shed, the larvae are exceptionally hardy". COLE's view that after liberation the food supply rather than temperature is the critical factor, is, however, only applicable to tank conditions. One of the principal advantages of spat-production in an enclosed basin is the increased measure of protection it affords to the larvae. Tidal currents and a great many animal enemies are thus shut out from the tanks. Consequently the length of the pelagic period has not much influence on the percentage of larvae that reach the setting stage. In the open sea, on the contrary, animal enemies and the tidal currents exact daily a heavy toll of the larvae, so that every protraction of the pelagic stage will cause a decrease in the percentage of larvae that reaches maturity. And although low water-temperatures do not directly cause an untimely death of oysterlarvae, they do bring about a protraction of the pelagic stage and thus become the cause of heavy losses.

As the protraction of the pelagic stage may be considerable (22° C: 7 days, 16° C: 16 days of pelagic life), it is my opinion that, although oysterlarvae are exceptionally hardy and can stand very low temperatures, the influence of the water-

temperature on the extent of the larval losses during the pelagic stage is very great.

Other abiotic factors are seldom considered as destructive agencies during the pelagic period. NELSON (1921) states that strong winds, which stir up large amounts of sediment, destroy many larvae: the larvae fill their digestion tracts with dirt instead of with their accustomed food, which may cause their death.

GAARDER (1932) states that a pH above 9.00 is injurious to oysterlarvae. Anorganic enrichment caused high pH values in the pollen. His experiments in vitro showed moreover that a copper content of more than 0,04 mg per litre is injurious to oysterlarvae.

Biotic destructive agencies

A great many marine animals that naturally feed on plankton-organisms exact a very heavy toll of oysterlarvae during the pelagic period. It is probable that practically all kinds of animals which strain the water for their food ingest oysterlarvae. Such animals can be divided in bottom-dwellers and sessile forms, such as barnacles, sea-squirts, other bivalves, certain worms, hydroids, etc., pelagic forms, such as most kinds of young fish, e.g. herring and anchovy, and some representatives of the macroplankton, such as *Aurelia aurita* and Ctenophora.

In several cases the ingestion of oysterlarvae has actually been observed and their presence in the stomach-content of various freshly-caught animals has been demonstrated.

Thus ORTON (1922 b) tells us that the jelly-fish *Aurelia aurita* greedily ingests larvae of *Ostrea edulis*, which are also to be found in the stomach-content of these animals when caught in the open sea. DODGSON (1922) describes observations made by SHERWOOD: *Noctiluca*, put together with young oysterlarvae (*Ostrea edulis*) in an aquarium, appeared to cause a rapid diminution in the number of these larvae. The actual ingestion of these oysterlarvae was repeatedly observed. HORI and KUSAKABE (1926) tell us that the larvae of *Ostrea gigas* are eaten by *Noctiluca* and by the larvae of Actiniae.

In some cases one kind of depredator is so numerous that but very few of the oysterlarvae can escape it. The spatfall

will be negligible in such cases. Thus NELSON (1925 a, 1925 b, 1927, 1929) tells us that there have been years in which myriads of the Ctenophore *Mnemiopsis leidyï* (Agassiz) decimated the larvae of *Ostrea virginica* in Barnegat Bay. As many as 125 early larvae were found in the stomodeum of a single specimen of this Ctenophore and as sometimes 15 Ctenophores occur in one cubic metre of water, the ravages caused by these animals may assume alarming proportions.

Other kinds of Ctenophores, such as *Pleurobrachia*, are likewise classed among the enemies of oysterlarvae (KINCAID 1915).

NELSON (1921) reckons the adult oysters among the depredators, for he found sometimes as many as 62 mature larvae in the stomach of one adult oyster. YONGE's (1926) classing the oyster as wholly herbivorous does not correspond with the facts (NELSON 1933), for *Ostrea virginica* ingests animal forms (Nematodes) as well and is able to digest them, too.

Although some authors assume that adults of *Ostrea edulis* likewise ingest a great many oysterlarvae (BIERRY and GOUZON 1939, CHAILLÉ 1938), the rightness of this assumption has not been sufficiently proved. I often examined the stomach-content of the adults of *Ostrea edulis* in the Oosterschelde in the course of the season of reproduction. Although I occasionally met with some oysterlarvae in the stomach-content, I never found such great numbers of ingested larvae as NELSON did in *Ostrea virginica*. It is my belief that other animals consume far more oysterlarvae than adult oysters do. Sea-squirts, barnacles and plankton-eating fishes abound in the Oosterschelde. *Noctiluca* is often very numerous here.

Especially in the summer of 1938 I noticed myriads of *Noctiluca* at the station Kattendijke. The Ctenophore *Pleurobrachia* is often numerous in the Oosterschelde. It is my belief that the toll levied by animal depredators is the most important cause of the diminution of the larval herds in the Oosterschelde. It is very difficult to estimate the part played by each of the various animal enemies.

Although the number of certain enemies is probably liable to annual variations, I have never yet found a sudden abundance of one kind of enemy causing a failure of the spatfall in the Oosterschelde. The daily toll exacted in the open sea is very heavy and every protraction of the pelagic period (e.g. by low water-temperatures) will decrease the percentage of the larvae reaching

maturity. This is the reason why high water-temperatures are so favourable to the propagation of oysters.

It is not possible to compute the exact percentage of the larvae that reach maturity, but as the number of mature larvae in 100 litres of water rarely exceeds a few tens, I believe that under normal conditions less than 10% of the larvae reach maturity in the Oosterschelde, probably even far less than 10%. If I estimate roughly from the data collected in my diagrams the percentage of the larvae that reach the full-grown stage, I find the following figures:

water-temperature	duration of the pelagic period	percentage reaching maturity
22° C	6– 7 days (13 tides)	10 %
20° C	10 days (19 tides)	5 %
18° C	12 days (23 tides)	2,5%

These figures, which are partly conjectural, enable me to calculate the daily losses (assuming that the larvae are about equally subject to devouring and dispersion during their entire pelagic life). After n tides the remainder is: $A (1— 1/p)^n$ (A: original number of larvae, $1/p$: decrease during one tidal cycle). Thus it can be computed from the figures in the table above that $1/p$, the loss during one tidal cycle, amounts to about 13 or 15%. As the water renewal causes a loss of about 4% during each tidal cycle, the remaining 10% are caused by other factors, probably mainly by animal enemies.

Consequently we may conclude that in the Oosterschelde, in consequence of favourable hydrographical conditions, the losses caused by the tidal currents amount to less than one third of the total losses. I do not doubt that this proportion will contrast favourably with that in many other breeding-grounds of *Ostrea edulis*.

XVIII. VERTICAL DISTRIBUTION OF FULL-GROWN LARVAE

Although it may be assumed that the oysterlarvae in the Oosterschelde are, generally speaking, uniformly distributed in a vertical sense (as has been discussed in a previous section), this does not imply that full-grown larvae, ready to settle, also show a uniform vertical distribution. Differences in the vertical distri-

bution of full-grown larvae will not appreciably affect the general vertical distribution, for the number of mature larvae of *Ostrea edulis* forms but a small percentage of the total number of oyster-larvae in the plankton.

Full-grown larvae are provided with a pigment-spot, which is often credited with a photosensitive character. It is often supposed that the pigment-spot enables the larvae to react to differences in light-intensity. Moreover, it is often assumed that mature larvae show a general inclination to make for the bottom regions when the moment of fixation draws near.

Not many observations have been made on the vertical distribution of full-grown oysterlarvae so far. MAZZARELLI (1922) who reared larvae of *Ostrea edulis* in vitro, tells us that larvae, on reaching maturity, cease hanging on the surface-film of the water: "Ad un dato momento sparice dal pelo dell' acqua lo strato formato dalle larve in posizione di riposo, le quali si sommergono del tutto e si mescolano alle altre." Very gradually "like a gentle rain", the mature larvae make for the bottom. The full-grown larvae, which continue swimming, are still uniformly distributed in the water: "E quindi lentissimamente, como una pioggerella sottile, le larve stesse cominciano a scendere al fondo. Le quali si accumulerabbero poi verso il fondo, ma pur restando sempre le larve regolarmente distribuite in tutta la massa acquea, il loro numero va scemando a poco a poco, man mano, che molto larve vanno e posarsi sul fondo, finchè rare restano le larve natanti."

COLE and KNIGHT JONES (1939) investigated the vertical distribution of oysterlarvae in tanks. They sampled in the daytime. Eyed larvae were abundant in the tank, but their distribution appeared to be similar to that of earlier larvae.

Not much is known about the vertical distribution of full-grown larvae of other kinds of oysters. NELSON (1931) states that his investigations have shown that mature larvae are much more abundant close to the bottom than in the upper layers, but he does not give any figures or details.

SEKI and TANAKA (1931) state that full-grown larvae of *Ostrea denselamellosa* are more numerous in the lower layers than at the surface of the sea. It is difficult, however, to infer the correctness of their statement from the figures in their tables. Both full-grown larvae and earlier larvae are more numerous in the bottom-samples, probably owing to differences in salinity.

Moreover the samples but rarely contain more than ten mature larvae, in most cases even far fewer. Such numbers are too small to base conclusions on.

I invariably measure all the larvae in the plankton-samples collected in the Oosterschelde. Moreover I note down which of them are provided with a pigment-spot. Consequently my special series of samples in particular enable me to find out whether or not full-grown larvae show a vertical distribution similar to that of earlier larvae.

The special samples were filtered off from 50 litres of water. I but rarely met with more than 10 full-grown larvae in these samples. As the bottom samples are collected about half a metre above the bottom, it is possible, though not probable, that an accumulation of mature larvae occurs in layers still closer to the bottom.

A series of samples collected at the station Yersche Bank (14 July 1939, fig. 11) seems to point to the possibility that mature larvae are relatively more abundant near the surface during the night and that they are distributed more uniformly in the day-time. In 9 couples of samples collected at this station during darkness I counted 46 full-grown larvae in the surface samples and 25 in the bottom samples. In 33 couples of samples collected at the same station in the day-time I counted 115 mature larvae in the surface samples and 127 in the bottom samples.

At Kattendijke I found in 4 couples of night-samples 42 mature larvae in the surface samples and 42 in the samples collected 5 metres below the surface. In 12 couples of day-samples I found 27 full-grown larvae in the surface samples and 25 in the samples procured from a depth of 5 metres.

Although these numbers are quite small, I believe that they suffice to justify the conclusion that full-grown larvae show, at any rate in the day-time, an uniform vertical distribution. I dare not decide whether mature larvae are really more abundant in the surface layers during darkness, as fig. 11 seems to indicate; the numbers are far too small to base conclusions on. Moreover at the station Kattendijke this phenomenon was not observed (fig. 12). In any case, I never noticed a marked accumulation of mature larvae in the bottom layers, which is in accordance with the data obtained by MAZZARELLI and COLE.

XIX. THE PROCESS OF ATTACHMENT

> "The setting period, though of relatively short
> duration in the development of the oyster, is of par-
> ticular importance, as at this time its existence as a
> sedentary organism begins, its future location is
> selected and its possibilities for survival are determined
> by the ability of the larva to obtain a favourable place
> for attachment." (PRYTHERCH, 1934 a).

In many accounts of the life history of the oyster the belief
is frequently expressed that fixation occurs when the shell
becomes so heavy that the larvae are no longer able to swim
continually and consequently sink to the bottom (e.g. MAZZARELLI
1922).

HAGMEIER rejected this view in 1916 already. COLE and
KNIGHT JONES assert that the old view is totally erroneous, as
the swimming powers of the larvae are undoubtedly greatest
during the period immediately preceding attachment.

The first part of this period, during which the larva searches
for a suitable substratum to attach, is called the searching stage
(PRYTHERCH 1934 a). At this stage the larva is swimming with
its foot protruding (COLE and KNIGHT JONES 1939: *Ostrea edulis*;
PRYTHERCH 1934 a: *Ostrea virginica*). PRYTHERCH observed that
the mature larva of *Ostrea virginica* often produces a thin
byssus-thread in the searching phase, in many cases of a consider-
able length. The entire setting behaviour may be interpreted
as an effort to find the ecological norm: i.e. the environmental
conditions that are necessary for their well-being (RUSSELL
1934).

NELSON (1931) says that oysterlarvae are positively stereo-
tropic during the searching period: that is they react strongly
to contact with surfaces, with a tendency to cling to them and
to crawl about on them.

YOKOTA (1936)[1] tells us how the full-grown larva of *Ostrea
gigas* reacts, when, descending, it comes into contact with an
object. It will descend about another 0,4 cm and will then rise
as high as the object which gave the stimulus and cling to it
or crawl about on it.

HOPKINS (1937) suggests that the chance of the foot touching
an object decreases according as the angle of surface of the object

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[1] I wish to express my thanks to Mr. S. Nakano of the Imperial Fisheries
Experimental Station for his kindness to translate Yokota's papers for me.

departs more and more from the "under horizontal", seeing
that in the normal swimming position of the larva of *Ostrea
lurida* (and of other species of oysters) the velum and the foot
project upwards, although HOPKINS admits that the foot is
extensible in all directions. HOPKINS puts forward this suggestion
in explanation of his observations on the influence of the angle
of surface on attachment, which phenomenon will be discussed
below. Mature larvae of other kinds of oysters swim in the same
way, but often show other reactions with regard to the angle of
surface; so it is my belief that HOPKINS' explanation is too simple.

The second part of the period immediately preceding attach-
ment is called the crawling phase. During the crawling phase
the larva crawls over the surface of the substratum with the
velum retracted, the foot extended in front and dragging the
shell. Crawling has been observed in *Ostrea virginica* (NELSON
1923 a, 1924 a, 1924 b, PRYTHERCH 1934 a), in *Ostrea edulis*
(COLE and KNIGHT JONES 1939) and in *Ostrea gigas* (YOKOTA
1936 b). Crawling may alternate with periods of normal swim-
ming. NELSON (1924 b) states that during crawling the larvae
describe circles of ever-decreasing diameter, but the other investi-
gators have not confirmed this statement. COLE and KNIGHT
JONES tell us that the larvae of *Ostrea edulis* crawl backwards
and forwards rather irregularly over a few centimetres of surface,
making "smaller excursions" in every direction. During the
crawling phase the foot of both *Ostrea edulis* and *Ostrea virginica*
gradually changes from a long slender shape to one that is short
and broad.

Both PRYTHERCH (1934) and YOKOTA (1936) state that during
crawling the larva stabilizes its body by sending out byssus.
These byssus-threads, which are at first cylindrical and about
0.004 mm thick (PRYTHERCH), prevents the larvae from being
washed off from the substratum during crawling. YOKOTA
describes how the larva of *Ostrea gigas* crawls towards a current
produced with a syringe. If the current is stronger some of the
larvae cease crawling and swim away, while others firmly
stabilize the body on the crawling surface. The body is supported
by the byssus and slightly rocks from side to side against the
current. If the current becomes still stronger the byssus snaps
off. This experiment can be repeated several times; it is the
simplest way to show the existence of a byssus-thread.

Such experiments have not yet been carried out on the larvae

of *Ostrea edulis*. COLE and KNIGHT JONES (1939) regard it as possible that such a fine byssus-trail is laid by the European oyster during the final phases of crawling. HORST (1884) tells us that he repeatedly observed fine threads, probably byssus-threads, attached to recently set spat. I also often observed such fine threads attached to the spat on my test-plates.

When crawling is finished, the real fixation takes place. Formerly it was generally assumed that the larva attaches itself to the substratum by starting the production of the disso-conch shell. It was assumed that to this end a contact was effect-ed between the secreting edge of the mantle and the substratum (HORST 1883, RYDER 1883, NELSON 1923 a, PRYTHERCH 1924: "by means of a shelly secretion of the left lobe of the mantle").

It was STAFFORD (1913) who found that the space between the left valve of the spat and the substratum was almost com-pletely filled with a cement -like substance. Anatomical investiga-tions of the fully developed larvae of *Ostrea virginica* led to his discovery of the byssus-gland. He came to the conclusion that this cement is poured out in liquid form from the byssus-gland. The same cement-like substance between the left valve of the shell and the substratum was found by HORI (1926) in *Ostrea gigas*. As to *Ostrea edulis* I frequently observed the occurrence of the cement between the left prodissoconch shell of the spat and the substratum; consequently it certainly is not only the new dissoconch shell which attaches the spat to the collector.

STAFFORD's assumption has been confirmed by the direct observations of NELSON (1924 b) and PRYTHERCH (1924) on *Ostrea virginica* and of COLE and KNIGHT JONES (1939) on *Ostrea edulis*. The cementing fluid is forced out of the byssus-gland by one or two vigorous contractions of the valves against the base of the foot. With the aid of the foot the left valve is pressed against the substratum in the place where the cementing fluid has been exuded. COLE and KNIGHT JONES (1939) tell us that the behaviour of the larva during the fixation act is curiously reminiscent of that of a dog preparing its beds: "The larva rocks the shell backwards and forwards and to some extent from side to side while, we suppose, the byssus cement is squeezed out, then comes to rest, and the shell twists over on its side, the left valve with to bigger umbo undermost, completing the setting process."

The cementing fluid hardens sufficiently in a minute or two

to prevent the newly-set spat from being washed off by a jet of water from a pipette (COLE and KNIGHT JONES 1939, PRYTHERCH 1934). According to NELSON (1924 b) the newly-set spat of *Ostrea virginica* is inclined to the surface of the substratum at an angle of about 30 degrees; according to PRYTHERCH (1934 a) at an angle of about 45 degrees. I observed that the angle of inclination between the spat and the substratum is about 45 degrees in *Ostrea edulis*. The growth of the dissoconch shell soon renders this angle of inclination less conspicious.

Both ERDMANN (1934) and COLE and KNIGHT JONES (1934) tell us that larvae, dislodged immediately after setting, are not observed to attempt to set a second time, although these larvae may remain alive for a considerable time. ERDMANN came across a few mature larvae with an empty byssus-gland. He assumes that these larvae are "over-ripe": "Allem Anschein nach ist somit die Sezernierungsfähigkeit der Drüse auf einen bestimmten Zeitpunkt beschränkt." With ERDMANN "maturity" of the larvae denotes a physiological phase: the ripeness of the byssus-gland.

His "beschränkter Zeitpunkt" is an elastic term, however, for COLE and KNIGHT JONES (1939) state that "crawling alternating with periods of normal swimming, may extend over several days, if no suitable surface for attachment is presented; this happens frequently if fully developed larvae are kept in clean smooth glass vessels without any other object to which they may attach. This ability to delay attachment, i.e. metamorphosis, is likely to be of considerable value in aiding survival of larvae carried to places where there are no suitable surfaces for attachment." It is the opinion of several American authors (PRYTHERCH 1929, MEDCOF 1939) that the full-grown larvae of *Ostrea virginica* settle without any considerable delay. The larvae from a single spawning appeared to attach within a period of two days at most. It is my belief that the enormous difference between the number of full-grown larvae and the number of successful spat (section XXII) justifies the conclusion that the period during which *Ostrea edulis* is able to attach is limited, which is in accordance with ERDMANN's view.

XX. THE ORIENTATION OF THE SPAT

> "It is probable that the settling down of pelagic larvae is a much more precise and complex affair than we imagine, involving specific behaviour-acts or trains of behaviour."   (RUSSELL, 1934).

In the course of my examination of the spat on my test-plates it struck me that the umbo of a high percentage of the spat points more or less in the same direction. This phenomenon has been noticed before by other investigators. Thus HORST (1883) tells us that the spat on his test-collectors was fixed with the hinge uppermost (*Ostrea edulis*). COLE and KNIGHT JONES (1939) state that the majority of the larvae of *Ostrea edulis* set with the dorsal margin (i.e. the hinge) uppermost on inclined or vertical surfaces.

PRYTHERCH states (1934): "When oyster larvae set upon a vertical surface, they invariably place the shell with the dorsal edge or hinge uppermost" (*Ostrea virginica*). YOKOTA (1936 b) tells us that on vertical surfaces it is normal for the spat to attach with the umbo to the left (posterior part turned upwards), when the spat is seen from the surface of setting (*Ostrea gigas*). Only transparent collectors allow us to see the spat from the surface of setting. On any other kind of substratum we shall see the spat from the opposite side. If he had observed it from this side, YOKOTA would have stated the umbos of the majority of his spat as turned to the right. Of 113 of his spats 70 umbos were thus turned to the right (posterior part turned upwards), 26 umbos pointed upwards, 10 pointed downwards and 7 were turned to the left (anterior part turned upwards).

My inquiries into this matter have shown me that these observations of YOKOTA hold good for *Ostrea edulis*, too. I noted down these data in a kind of compass-card. The direction "North" indicates the side of the test-plate that was uppermost during attachment (i.e. the projection of the vertical).

YOKOTA's data visualised in this manner yield the following diagram:

I used eight sectors instead of four, however. I have analyzed the orientation of the spat I found on the test-plates that I used for studying the effect of the angle of surface:
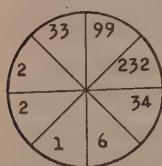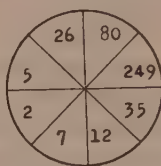
| angle of surface 90° | angle of surface 112½° | angle of surface 67½° | angle of surface 135° |

| angle of surface 45° | angle of surface 157½° | angle of surface 22½° |
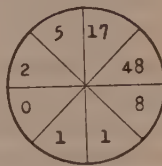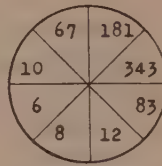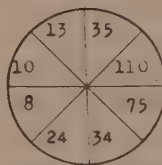
These data clearly show that the larvae of *Ostrea edulis* prefer to attach with the umbo pointing in a definite direction. This direction is not simply "upwards" or "to the right", as the above-mentioned authors believed, although the majority of the umbos certainly point to the right as well as upwards.

The direction they prefer apparently forms an angle facing to the right of about 30° with the horizontal axis. In other words: there is an angle of 60° between the direction of preference and the projection of the vertical (direction "North"). Not all the larvae succeed in attaching in the preferred direction; a certain percentage point in other directions, but for the greater part in adjacent directions and but seldom in the opposite direction. The distribution over the compass-card is remarkably similar on test-plates that have been exposed at angles of surface of 90°, 112½°, 67½° and 135°. For the sake of uniformity I have adopted the notation of angles of surface used by other investigators. The upper surface of a plate inclined at an angle of 45° is considered as being at an angle of 135° to the horizontal and other surfaces accordingly. When the angle of surface is 157½°, the distribution is essentially the same, but a greater percentage

of the umbos point in the adjacent directions and the number that point in the opposite direction is somewhat greater, too. In cases where the angle of surface was 45° and especially in those of 22½°, the percentage that point in the opposite direction is still greater, while moreover the angle of preference appears to be somewhat smaller than 30°, as the adjacent sector situated under the horizontal axis (E.S.E.) shows more spat than the sector (N. N.E.) placed immediately above the sector of preference (E.N.E.).

Moreover I analyzed the spat on many test-plates that had been exposed horizontally. As will be discussed below, these test-plates have been placed in containers of reinforced concrete. These containers are placed on the seabottom. It was not possible to place such a container in a precisely horizontal position. So the "horizontal" test-plates have not been exposed in a precisely horizontal position, but at slight angles in an unknown direction.

The oysterlarvae are apparently extremely sensitive to the angle of surface, for although the deviation from the horizontal will have been very slight in most of these cases, the analyses of the spat practically always show which side of the test-plate has been uppermost. The more equal the distribution over the compass-card, the slighter the deviation from the horizontal will have been. The side which has apparently been uppermost is indicated by an arrow (60 degrees to the left from the direction of preference).
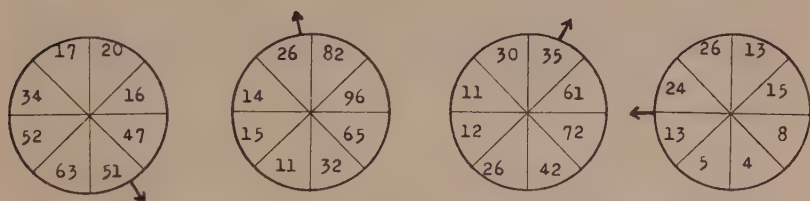
I herein reproduce the compass-cards of two cases which did not show a distinct direction of preference. The test-plates in question will have been exposed at an angle, deviating little from the horizontal:

The pull of gravitation is apparently the only factor that governs this remarkable orientation.

This apparent influence of gravitation on the setting process is not in accordance with NELSON's view (1921) that the pull of gravitation has practically no influence in determining the position of attachment of oysterlarvae. Whether in this respect *Ostrea virginica* reacts in the same way as *Ostrea edulis* still remains to be seen, however.

Test-plates exposed both in light and darkness show the same phenomenon. Another series of experiments provided me with test-plates along which the tidal currents swept from one and the same side during the entire period of exposure. The orientation proved to be the same whether the current came from the "left" (direction W) or from the "right" (direction E).

It is very remarkable that the orientation is essentially the same on surfaces inclined at an angle of 0 to 90° and on those inclined at angles of 90 to 180°, although the axis of the spat points in quite different directions in all these cases.

XXI. METHODS OF DETERMINING THE INTENSITY OF SETTING

No data on field-observations on the intensity of setting throughout the season of reproduction in *Ostrea edulis* are available. COLE (1939) measured the frequency of setting in tanks (so under semi-natural conditions) by exposing smooth slates for 24 hours. Afterwards the spat was counted with a binocular microscope.

More is known about the intensity of setting in other species of oysters. It is my opinion, however, that the methods generally used cannot exactly be called smart.

Gryphaea angulata

CHAUX-THÉVENIN (1933, 1934) immersed some test-collectors every fortnight. He did not compare the numbers of spat of *Gryphaea angulata* on these collectors till the next year. As the percentage of the newly-set spat surviving till the next year is but small and moreover not always the same, this method can hardly be called quantitative.

Ostrea virginica

NELSON (1923 b, 1924 b, 1927, 1928 a, 1930, 1931) determined the time and intensity of setting by placing two shells in a wire basket, which shells he intended to replace by a new couple every 24 hours. In practice, however, the period of exposure was almost invariably considerably longer than 24 hours. The shells were not exchanged very regularly. Although the outer surfaces of the shells may frequently bear a good deal of spat, NELSON "for ease and accuracy" only counted the spat in the concave or inner faces.

It is my belief that shells are not suitable for the purpose of determining the intensity of setting quantitatively. As will be discussed below the angle of surface has a great influence on the frequency of setting. Both the irregular shape of the shells and the impossibility of exposing them every time in exactly the same position prevent a reliable measuring of the spatfall. NELSON placed one of his shells with the inner surface upwards and the other downwards, but I do not believe that this is an effective method of getting round this difficulty. PRYTHERCH (1929) and HOPKINS (1931) used wire bags with shells to determine the frequency of setting, while MEDCOF (1939) used one single shell, which he exposed for 24 hours.

Later on PRYTHERCH (1934 a), in a special experiment, used a more quantitative method. He immersed cement-coated partition-collectors at regular intervals. He allowed the spat to grow somewhat before he proceeded to counting. Errors owing to the influence of the angle of surface need not be feared with this device.

Ostrea lurida

COE (1932 b) and COE and ALLAN (1937) obtained some data on the season of attachment of Ostrea lurida in the course of their studies on sedentary organisms.

Both BONNOT (1936, 1937 a) and HOPKINS (1937) used wire bags filled with shells of Ostrea gigas to determine the intensity of setting. The shells remained in the water for 7 days. HOPKINS used two series of bags at each station, so that the one series overlapped the other. The spat on the inner surfaces of the shells was counted with a binocular microscope. The outside of the shells is too rough and lamellate for the attached spat to be counted with sufficient accuracy. According to these investigators

the inner surfaces carry on an average about 30 to 35% of the total number of spat on the shell. The differences between the shells from one bag in the number of spat caught on the inside surfaces were enormous, which is obviously due to differences in the angle at which the shells were held, as well as to their size and their position in the bag. I do not believe that HOPKINS is right in assuming that any error traceable to the angle of surface is eliminated by the fact that the shells are held at every possible angle in the bag.

The method of determining the intensity of setting used in the Oosterschelde has been described briefly in HAVINGA's papers (1938, 1939). Since 1935 the periodicity of the spatfall has been determined here throughout the seasons of reproduction. In 1935 two spatfall-stations were established, in 1936 five, of which the stations Yersche Bank and Kattendijke are the most important for our purpose, seeing that the daily plankton-samples are procured at these stations. The spatfall is measured by regularly exposing sets of 3 specially prepared glass plates for periods of 3 days at each station. The plates are placed in containers of reinforced concrete, weighing about 40 kg, of which the side-walls have oblong narrow holes let into them (fig. 16). The plates are held in the containers at angles of 45°. A regular record has been kept of the places in the container in which the plates have been exposed and of the sides that have been uppermost. To keep off floating sea-weed the entire container is covered with wire-netting. The containers are placed on the sea-bottom in places which are not exposed during low water. The plates, which measure 13 × 18 cm, are of glass ground on both sides. The grounding causes the coating to adhere firmly to the plates. The thin coating is composed of a mixture of equal parts of cement, lime, fine sand and water. This coating provides the oysterlarvae with an excellent substratum to attach on.

Before the prepared plates can be used it is necessary that they should have been dried thoroughly. When the plates have not been dried long enough, they will get covered with a thin slimy layer when exposed in seawater; this layer catches many sand-particles and such plates are unsuitable as collectors. The same phenomenon is observed when limed tile-collectors have not been thoroughly dried. French oyster farmers speak of such tiles as "caillée". If there should be too little time for thor-

Fig. 16. Container for the determination of the intensity of the spatfall. (Photo Havinga).

Fig. 17. Floating container for the growing up of the spat. (Photo Havinga).

Fig. 18. Container with horizontal and vertical plates. (Photo Havinga).

Fig. 19. Container for 12 plates. (Photo Havinga).

ough drying, bathing in a solution of sodium-bicarbonate may be substituted as a way of eliminating this drawback (CO_2!).

After the plates have been exposed in the container for three days they are replaced by another set. The plates are carried in a zinc container, filled with seawater, to an oysterpit near the laboratory at Bergen op Zoom.

As the counting of the spat with a microscope is very time-devouring, we let the spat grow for another 6 days, so that they can easily be detected with a lens magnifying 6 times. For this purpose the plates are placed for 6 days in a wooden box floating in an oysterpit (fig. 17). To keep off further oysterlarvae and enemies and to admit organisms on which the newly-set spat can feed two of the side-walls of the box have been made of bolting silk, which is regularly exchanged and cleaned. It appeared to me that practically none of the newly-set spat died during this 6 days' period. At the end of it the plates are carried to the laboratory, where they are exposed to the air to dry. When the plates are dry, the counting of the spat may be proceeded to at any time. The shells of the spat measure from about 0,6 to 0,8 mm at the time they are dried. This size is reached in 6 to 9 days of growth after attachment. The largest specimen often reach 1,0 mm in 9 days of sedentary life.

If the plates are exposed for 6 days, which is often the case with special series of plates, the oldest spat will be about 12 days when they are taken from the water. The largest spat on such plates measure from 1,2 to 1,4 mm.

The young spat is easily recognizable. Sometimes the plates will also be found to carry small mussels (*Mytilus edulis*) and small specimens of *Tapes pullastra*, which differ not only in size and colour from oyster-spat, but which are moreover attached by means of byssus-threads only and can consequently easily be moved to and fro with a needle.

Sometimes profuse settings of barnacles will be found to occur on the plates. The intensity of the setting of barnacles showed marked maxima and minima.

The spat is counted with the aid of a large kind of counting-table on which the plates are moved in a systematical manner under a lens, which is held in a support.

Special series of plates used in other experiments on the spatfall are treated in the same way. The plates can be used

more than once; they can be cleaned by scrubbing them with an abrasive.

XXII. THE CORRELATION BETWEEN THE NUMBER OF FULL-GROWN LARVAE AND THE INTENSITY OF SETTING

Although it is self-evident that the presence of full-grown larvae is a conditio sine qua non for setting, many authors studying the problems of spatfall omit to investigate the number of full-grown larvae quantitatively.

The waves of setting are determined by an optimum combination of certain environmental factors. The suitability of a particular place for spatfall is dependent on several factors, as will be discussed below. These factors may cause enormous differences in the suitability for collecting oysterspat. Though the number of full-grown larvae in different places, with different environmental conditions, may be the same, this does not imply that the number of spat caught will also be the same. The intensity of setting in one and the same place, however, is correlative with the number of full-grown larvae present there. In other words: the proportion between the number of full-grown larvae and the intensity of the spatfall is fairly constant for each particular place, but the value of this ratio may be different for each place.

I share KÄNDLER's view (1928) that it is not sufficient to count the total number of oysterlarvae in the water on investigating the periodicity of setting, but that it is also necessary to measure the larvae and thus to determine the number of full-grown larvae.

The French investigators do not measure the larvae, but they divide them in larvae in the first stage (straight-hinge larvae) and larvae in the second stage (umbo larvae). BOURY (1930) suggested a comparison of the number of larvae in the first stage with the number in the second phase, so as to get an idea about the average degree of development of the larvae. It was BORDE (1931, 1932, 1935, 1936, 1937) who established the "fixation-coefficient":

$$\frac{\text{number of larvae in the second stage}}{\text{total number of larvae}}$$

This "fixation-coefficient" is assumed to indicate what percentage of the larvae approaches the setting stage. It is my belief,

however, that it is not the percentage of full-grown larvae which is proportionate to the intensity of setting, but the number of full-grown larvae per unit of water. BORDE (1935) tells us that in the basin of Arcachon the total number of larvae decreases according as the samples are collected at greater distances from the centre of larvae-production, but that the "fixation-coefficient" increases proportionately in such series of samples. The same phenomenon may no doubt be observed elsewhere, for instance in the Oosterschelde. In the centre of larvae production the number of newly-liberated larvae is greater than that at other stations. Assuming that the number of full-grown larvae is about the same at the various stations, the "fixation-coefficient" will be by far the smallest in the centre of larvae-production! The spatfall prospects are, however, certainly not least favourable in the centre of larvae-production! If the number of umbo-larvae remains constant for some days at a particular station, and with it the spatfall prospects, a new wave of swarming may cause a considerable decrease of the "fixation-coefficient"! Therefore it is my belief that this „fixation-coefficient" is of no use in the study of the intesity of the spatfall. Though high values of this coefficient will often be followed by a heavy spatfall, its application is no safeguard against disappointments (HERMAN 1938 a, LADOUCE 1938 a).

If we want to know something about the spatfall prospects, we shall have to determine the number of full-grown larvae (if desired: umbo-larvae) per unit of water.

NELSON (1927, 1928 a, 1929, 1930) visualized in his diagrams by means of solid black circles the samples in which mature oysterlarvae (*Ostrea virginica*) formed a portion of the total catch. The number of mature larvae per unit of water has not been recorded by NELSON, however.

His diagrams show that the duration of setting closely paralleled the period during which eyed larvae were found in plankton-collections.

PRYTHERCH (1929, 1931) collected a series of samples (each sample from 200 gallons of water) throughout a tidal cycle. The majority of the oysterlarvae (*Ostrea virginica*) in these samples were found to be at later stages of development and within a few days of setting. Roundabout low water he counted about 100 larvae in his samples, during the remainder of the tidal cycle less than 10. PRYTHERCH measured the spatfall by immer-

sing cement-coated partition-collectors from hour to hour. The collectors exposed roundabout low water caught far more spat than the others, which fits in with the data on his plankton-samples.

SEKI and TANAKA (1931) conclude that the intensity of setting of *Ostrea denselamellosa* depends upon the number of full-grown larvae, for they found that the time at which full-grown larvae abounded was the most suitable moment to place collectors.

The diagrams (fig. 7, 8, 9, 10) on the data obtained in the course of my investigations in the Oosterschelde (in the years 1936, 1937 1938 and 1939) show both the number of larvae in the different size-classes per 100 litres of water and the number of spat collected on one plate (i.e. the total number caught by the entire set divided by 3) at the same station in periods of 3 days. The correlation between the number of full-grown larvae and the intensity of setting strikes the eye, especially if the left part of the diagram is covered by a piece of paper, so that only the data on the largest size-groups (from 24 × 11 μ to 27 × 11 μ) and those on the spatfall remain visible. It is interesting to note the close parallelism between the intensity of setting and the periods during which full-grown larvae abound.

Very striking are the events in the month of August 1938. High water-temperatures favoured the growth of the larvae to such an extent that the numbers of full-grown larvae counted in the samples from 5 to about 14 August were unprecedentedly high. Consequently the spatfall was very profuse during the same period. A slight peak in the number of mature larvae in the second part of July in the same season corresponds with a slight increase of the spatfall shortly after. In 1939 the greatest numbers of full-grown larvae as well as most of the spatfall were recorded in the middle of July. In 1937 it was the second part of July that yielded the largest numbers.

Both the station Yersche Bank and the station Kattendijke show this correlation between the number of full-grown larvae and the intensity of setting. The diagrams clearly show, however, that the proportion between the number of full-grown larvae and the number of spat is not same at these two stations. The same number of mature larvae yields far more spat at the station Yersche Bank than at the station Kattendijke. It should be remembered, however, that the number of larvae is fairly constant in the course of the tidal cycle at the station Yersche Bank, while the number of larvae at the station Kattendijke

shows its maximum roundabout low water (the daily samples are taken at low water there). Moreover there are still other factors which influence the proportion between the number of mature larvae and the intensity of setting at different stations, as will be discussed below.

It is not easy to estimate what percentage of the mature larvae succeed in accomplishing attachment.

BIERRY and GOUZON (1939) estimate that about 400 out of 1 000 000 newly-liberated larvae attach on an average.

It is interesting to estimate the proportions between the number of young larvae, mature larvae and spat in the Oosterschelde. The following are the figures for the year 1939 (partly conjectural):

The number of adult oysters in the Oosterschelde was about 36 000 000 in 1939, while the percentage of functional females in the course of the season of reproduction may be estimated at 75% to 100%, as has been discussed above. The number of larvae produced by one oyster in the female stage varies from 500 000 to 1 000 000. Hence the total production of larvae in 1939 may be estimated at 10 000 000 000 000 at least. This figure is certainly not too high, for I very often counted from 200 to 300 larvae in 100 litres of water at a station situated far from the centre of larvae-production (Kattendijke). From this it follows that the water that fills the basin during high tide (675 000 000 m³) contains more than one larva per litre, which comes to more than 1 000 000 000 000 larvae in the entire basin on one particular day!

How many larvae reached the full-grown stage in the Ooster-schelde in the course of 1939?

On several days the number of full-grown larvae exceeded 10 per 100 litres (fig. 10) at both stations, which corresponds with at least 50 000 000 000 full-grown larvae in the entire basin on one particular day. I believe that the total number of full-grown larvae in the course of this season of reproduction may be estimated at 250 000 000 000 at least (i.e. 2,5% of the original number of larvae). In a previous section I estimated the percentage of larvae reaching maturity in the Oosterschelde at less than 10%, probably even far less. Only in periods charac-terized by very favourable water-temperatures, such as the middle of August 1938 and July 1935, does the percentage probably exceed 10%.

I examined the number of spat on a good many samples of tile-collectors and of sown-out shells in the autumn of 1939. Knowing the total amount of collector-material, I estimate that the number of spat caught in the Oosterschelde in 1939 and surviving till in October amounted to at least 250 000 000.

Perhaps only 1 out of 10 newly-attached spat survives till in October. The other 9 perish, partly by the action of animal enemies, partly by smothering. The most notorious enemies are *Carcinides maenas* and *Asterias rubens*. Small specimens of the latter sometimes occur in great abundance on the tile-collectors in some places. Smothering is caused by a rapid growth of other sessile invertebrates (e.g. *Ascidiella*, *Botryllus*, Sponges and Bryozoa) or by the deposition of silt or sand. I base the proportion 1 out of 10 on the following facts: The surface of the collectors is only for a limited period suitable for fixation. Soon a thin slimy layer, formed by a growth of microscopical algae and bacteria, renders attachment impossible. It is my belief that the suitable period does not exceed 12 days. My test-plates, which were exposed in the most favourable part of the season in 4 successive periods of 3 days, at two stations where many tile-collectors are placed as a rule (Wemeldinge and Kattendijke), caught from 100 to 150 spat in the aggregate. The surface of a tile-collector is about 8 times as large as that of a test-plate. So I assume that the number of spat caught by one tile-collector during the most favourable part of the season 1939 may be estimated at 500 or 1000. On many tiles from these stations I counted from 50 to 100 spat in October, which shows that about 9 out of 10 spat had disappeared. This proportion is of course subject to fluctuations in different years.

As at least 250 000 000 spat survived till in October, the total number of larvae that accomplished fixation will have been at least about 2 500 000 000. So we may say that about 250 out of every 1 000 000 liberated larvae settled. This number does not differ essentially from the estimate by BIERRY and GOUZON (i.e. 400).

When we compare the number of settled spat with the number of full-grown larvae in the course of the season of reproduction, we shall find that only about 1 % of the mature larvae succeed in accomplishing fixation!

Though my figures are partly conjectural, I am of opinion that indeed but a small percentage of the full-grown larvae

succeed in finding a suitable collector and in attaching on it.
This is probably due to lack of collector-material. The surface
of the collectors is only for a short period in a condition suited
to fixation. Moreover there are vast areas where the larvae,
which are at the mercy of the tidal streams, practically never
meet with any suitable piece of collector-material.

Interference on the part of man may effect an increase in
the percentage of mature larvae that accomplish attachment.
To that end the oysterfarmer will have to lay out his cultch
in adequate amounts in suitable places and at the most propitious
moment. Nature largely governs the values of the other propor-
tions and percentages.

The smallness of the percentage of mature larvae accomplishing
fixation is the reason why the number of spat per collector does
not perceptibly decrease when collectors are planted together
in great numbers in one place, if only care is taken that all of
them are easily accessible for the larvae-bearing water. In my
experiments there was no perceptible difference between the
number of spat per plate in containers with 3 plates and in those
with 12 plates, placed at the same station. The oysterfarmer in
placing his collectors need not take into account that" where
the tiles are many, the spatfall is poor".

From the foregoing it may be concluded that the "useful effect"
is not very great in the propagation of *Ostrea edulis*. Only about
25 out of a million larvae are found back on the collectors in
autumn.

This percentage of survival is not only small when considered
by itself, but also when compared with the results in the propa-
gation of other kinds of marine Molluscs. SMIDT (1938), for in-
stance, estimates that about one eighth of the larvae of the
Gastropod *Rissoa membranacea* succeed in accomplishing meta-
morphosis!

XXIII. THE EFFECT OF TEMPERATURE AND SALINITY ON THE SETTING PROCESS

LEENHARDT (1922, 1924) assumed that mature oysterlarvae
are incapable of attachment, unless the temperature of the
water is at least 18° C: "La fixation ne devant s'opérer que si
la température était d'au moins 18°". MAZZARELLI (1924),
however, assures us that in the lake of Fusaro fixation is certainly
possible at temperatures below 18° C. Later French investiga-

tors (e.g. Boury 1928) did not succed in establishing a tempera-
ture-limit below which attachment is impossible (field-observa-
tions).

Cole (1939) observed that fixation in his tanks was not
affected by sudden falls in temperature. He describes several
cases in which a sudden drop in water-temperature had no visible
effect on the larvae that at this time were on the point of setting.
Under tank-conditions no correlation between temperature and
intensity of setting can be established.

I fully suscribe to Cole's view. A comparison of the number
of full-grown larvae and the intensity of setting in the Ooster-
schelde has led me to the conclusion that fluctuations in water-
temperature do not affect the value of this proportion. Although
stormy spells of weather frequently occurred, often accompanied
by sudden drops in water-temperature (e.g. 22–26 July 1937,
early in July 1938, 12–22 August 1938, early in August 1939),
they produced no visible effect on the correlation between the
number of full-grown larvae and the intensity of setting.

Though the intensity of setting was very slight in the first
part of July 1938, owing to the small number of mature larvae,
the diagram clearly shows that the proportion between the
number of mature larvae and the number of spat caught on
the plates was essentially the same during the stormy days early
in July and in the subsequent calmer period.

It is my belief that the process of attachment is practically
not liable to varying water-temperatures and stormy weather
(of course only as far as temperatures recorded in the field in
the summer-months are concerned). This is in accordance with
Hopkins' view (1937) that "local weather conditions appear to
have little or no influence upon the setting of larvae, save in
their effect upon water-temperature which controls spawning
and rate of larval development."

It has been supposed by some that salinity influences the
intensity of setting. In the Southern parts of the range of *Ostrea
virginica* (Louisiana, Galveston Bay) fluctuations in salinity are
often considerable. Moore and Pope (1910) and Moore (1913)
observed no spatfall during the period in which the crevasse
water from the river Mississippi was pouring over the beds.
Hopkins (1931) concluded that setting periods coincided with
increases in salinity above about 20 parts per 1000, although no

exact limit could be stated. He assumes that "completion of larval development is not attained, unless the water reaches these higher concentrations." As no exact data on the number of mature larvae have been provided by these authors, we cannot be sure whether this influence of salinity is due to an insufficient development of the larvae or to failure in fixation.

PRYTHERCH (1934 a) ascertained the time required by the larvae to complete the setting process under various salinity conditions (experiments in vitro). The larvae of *Ostrea virginica* were found to complete fixation in 12 to 19 minutes when the salinity was 16–18,6 $^\circ/_{\circ\circ}$. Higher or lower salinities appeared to protract the setting process (the limits were: 5,6 $^\circ/_{\circ\circ}$: 140 minutes; 32,2 $^\circ/_{\circ\circ}$: 144 minutes). PRYTHERCH ascribed the effect of salinity on the setting process to a physical change in the byssal fluid, in consequence of which the time required for its complete discharge is altered.

Observations in Long Island Sound (HIGGINS 1938) have shown that the salinity of the bottom water there is only liable to slight seasonal changes, so that this factor cannot be held responsible for the success or failure of the spatfall.

The same conclusion may be drawn from the data concerning the Oosterschelde. The salinity in the Oosterschelde is only subject to slight seasonal changes in the year's course and remains virtually the same from year to year (table 2, fig. 3). So this factor cannot be held responsible for the success or failure of the spatfall in this case either. Any difference in the proportion between the number of full-grown larvae and the intensity of setting, owing to changes in salinity, cannot be shown here. Experiments in vitro, like those of PRYTHERCH, have not yet been carried out with the larvae of *Ostrea edulis*, but it is an established fact (MAZZARELLI 1924) that fixation of *Ostrea edulis* is certainly possible with salinities of even 34 to 39 $^\circ/_{\circ\circ}$.

XXIV. THE INFLUENCE OF COPPER ON THE SETTING PROCESS

PRYTHERCH (1931, 1934 a) ascertained the intensity of setting in the course of the tidal cycle in Milford Harbor (*Ostrea virginica*). The setting there appeared to be by far most abundant round-about low water. He concluded: "It is evident, therefore, that attachment of the larvae is not a haphazard process, but is

a definite biological reaction in response to some environmental stimulus." PRYTHERCH ascribed the tidal periodicity in setting to differences in the chemical composition of the water in the course of the tidal cycle.

The salinity appeared to show considerable differences at different stages of the tide, owing to inflowing river-water. The river-water contains in relatively large amounts several elements which are rather scanty in sea-water. Consequently these elements show their maximum at about low water at his station. PRYTHERCH assumes that the presence of adequate amounts of one of these elements is required to initiate the process of attachment. He came to the conclusion both by laboratory experiments and field observations that the required element is copper. He presumes that copper dissolved in river-water is precipitated in the form of copper-oxychloride when the river-water is discharged in the sea. According to PRYTHERCH fine particles of this precipitate are ingested by mature oysterlarvae. He supposed that the larvae cannot complete attachment, unless adequate quantities of copper have been ingested by them.

PRYTHERCH tries to prove the correctness of his assumption by pointing to the neat parallelism between the course of a graph on the intensity of setting and one on the copper content of the water for the same station. (fig. 20).

Personally, I do not agree with PRYTHERCH's interpretation.

In the first place it is the chemical side of the question that is liable to exception. Ir. F. LIEBERT, Director of the Governmental Institution for Chemical, Microbiological and Hydrographical Fishery Research informed me that the few reliable determinations of copper in sea-water yielded from 0,005 to 0,01 mg per litre. PRYTHERCH's figures are very high; they amount from 0,25 to 0,50 mg per litre at low water. A reliable determination of copper with sodiumdiaethyldithiocarbaminate is only possible after the element iron, which is always contained in relatively large amounts in river-water, has been eliminated. PRYTHERCH does not describe if and how he eliminated iron and he does not tell us whether or not he filtered his samples of water. Reliable determinations of the copper-content in coastal water have not been carried out so far.

In the second place it is the biological aspect that is subject to exception. I doubt whether oysterlarvae really ingest particles of copper-oxychloride. Some preliminary experiments with young
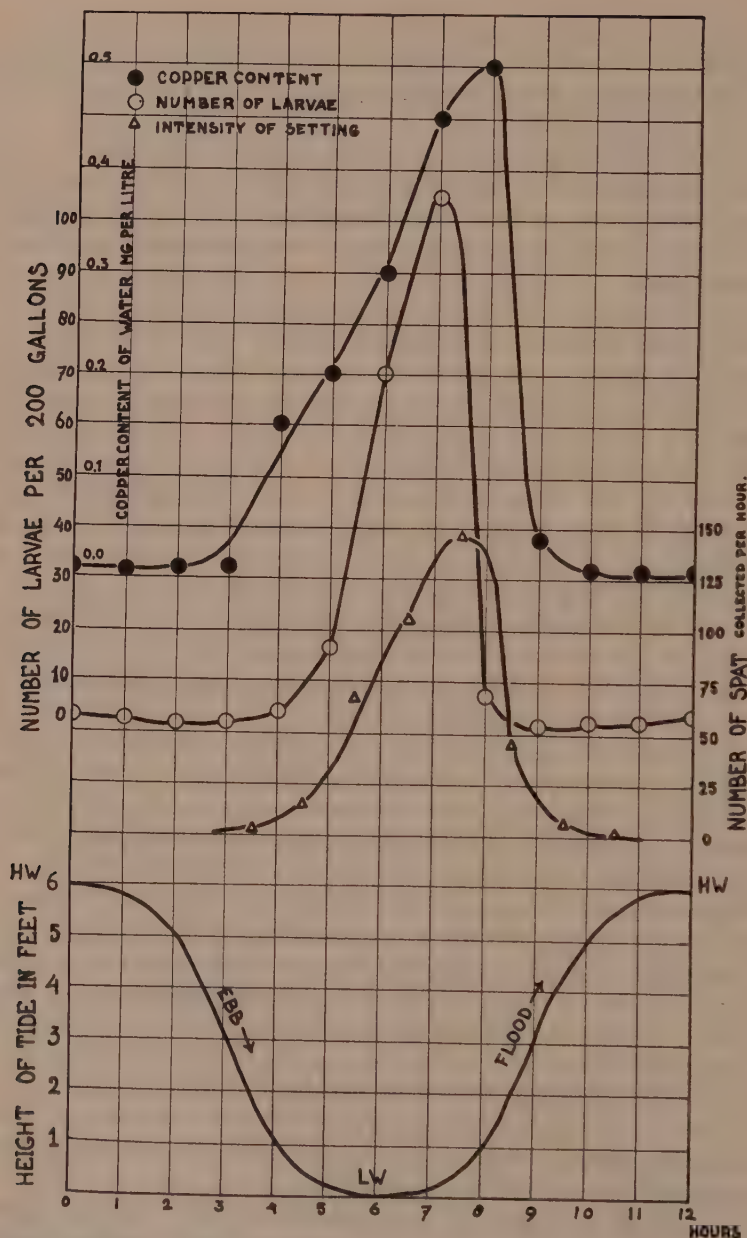
Fig. 20. The influence of copper on the setting process. After PRYTHERCH, altered by me. The graph on the number of larvae has been traced by me.

oysters and mussels have shown us that they do not ingest copper-oxychloride. The particles of copper-oxychloride are rejected be them as pseudo-faeces. In a little time they close their shells and stop feeding.

PRYTHERCH does not describe in his papers how he expects his mature larvae to swim. If the larvae are carried along with the currents, they will remain in the same body of water during the entire tidal cycle, so that they are not subject to differences in the chemical composition of the water in which they swim. It is only when they remain in the same place throughout the tidal cycle that at low water they may find themselves in water of another chemical composition than at high water. PRYTHERCH's views on the vertical distribution of the larvae have so far received no support whatever, as has been discussed above.

PRYTHERCH neglects one of the most important factors governing the intensity of setting, i.e. the number of full-grown larvae per unit of water! If there is any factor influencing the intensity of setting during a certain part of the tide, it is to be expected that the proportion between the number of mature larvae and the frequency of setting will vary in the course of the tidal cycle. The determination of the intensity of setting is not sufficient by itself to prove the influence of such a factor. In a previous paper (1929) PRYTHERCH stated the number of larvae (which practically all of them happened to be ready or nearly ready to settle) per 200 gallons of water in the course of the tidal cycle. This number showed a marked peak at low water. I am inclined to ascribe the differences in the number of his larvae to an influence of the currents on their distribution. PRYTHERCH gives no details about the currents, however. In his diagram (1934) I traced a graph on the number of larvae recorded in the above-mentioned paper (fig. 20), taking as my starting-point the average number of larvae per 200 gallons in 3 series of his samples, collected in 3 successive days (PRYTHERCH 1929). This graph closely parallels that on the intensity of setting! It shows that the proportion between the number of mature larvae and the intensity of setting remained constant in the course of the tidal cycle. This proves that other factors, such as the copper content, had no influence on the intensity of setting!

From all this it will be clear that PRYTHERCH's original diagram cannot be used in support of his assumptions!

I do not mean to say that oysterlarvae may not require some copper in the course of their development. I only want to demonstrate that PRYTHERCH's conclusions have not been borne out by the results of his field-experiments. His conclusions that the location of natural oysterbeds depends on the local occurrence of adequate amounts of copper and that failures in tank-breeding can be ascribed to a deficiency of copper, are very premature.

Nor have PRYTHERCH's assumptions been supported by experiments carried out by other investigators. NELSON (1931) tried to obtain a more intensive setting by adding copper to the cultch-material. Neither in Barnegat Bay nor in Delaware Bay was there any evidence, however, that the treatment of cultch with copper salts or the addition of metallic copper served to increase the spatfall. Basing himself on PRYTHERCH's conclusions, HOPKINS (1937) ascribed a small difference in the vertical distribution of the spatfall of *Ostrea lurida* to very slight differences in salinity (i.e. in the chemical composition of the water). I believe, however, that there is a simpler way of accounting for these differences in spatfall, for which it would be necessary, however, to measure the current velocities and to count the larvae.

GAARDER (1932, 1933) considered the possibility of a deficiency of copper in the water of the enclosed Norwegian pollen. He added copper to the water of one of the pollen, but he did not observe a clearly marked difference between the intensity of setting in the treated poll and in a neighbouring poll to which no copper had been added.

VOISIN (1933) does not absolutely deny that some copper may be required by oysterlarvae. If it should be required, he thinks it very probable that coastal waters contain this element in sufficient quantities. This is also my view of the matter. I have never yet made any field-observation which showed that copper affects the spatfall in the Oosterschelde. As the salinity of the water in the Oosterschelde remains constant in the course of the tidal cycle, and along with it the content of chemical components, it is impossible to ascribe periodicities in spatfall here to an influence of copper on attachment.

XXV. THE CORRELATION BETWEEN THE PERIODICITY IN SWARMING AND THE PERIODICITY IN SETTING

The importance of a sufficient knowledge of the events in the course of the pelagic life of oysterlarvae is often underestimated. In previous sections we discussed how variations in environmental conditions will affect the percentage of larvae that reach maturity.

Several authors neglect the influence of the lot that befalls the larvae and assume that the periodicity in setting is proportionate to the periodicity in swarming. Thus ORTON states (1937 a): "The density of the subsequent fall will be proportional to the percentage of blacksick oysters if healthy growth and settlement occurs."

HOPKINS (1937) carefully ascertained the frequency and intensity of spawning in *Ostrea lurida* by opening oysters periodically. HOPKINS made no quantitative investigations on the number of larvae in the water, for he considered this superfluous in case the periodicity of spawning is precisely known. In his discussion of the correlation between spawning and setting he has to admit, however, that the record of setting resembles that of spawning in some respects only. He regards considerable differences in mortality of the larvae of the various spawning maxima as a possible explanation of the imperfect resemblance between his graphs on spawning and setting. As he did not have at his disposal any data on the course of events in the long period of pelagic life (about 30 days in *Ostrea lurida*), he was unable to demonstrate the occurrence of such differences in mortality.

PRYTHERCH (1929) ascribes success or failure in setting of *Ostrea virginica* entirely to success or failure in spawning. According to him the quantities of eggs and sperm developed annually by adult oysters govern the intensity of setting. He assumes that the extent of spawning and with it the extent of setting are governed by temperature conditions. He shows that in those summers between 1922 and 1927 that were characterized by a successful setting the air-temperatures were above the normal. Although his conclusion that the warmest summers yield the best spatfalls holds good for the Oosterschelde as well, as will be shown below, I shall demonstrate that here it certainly is not mainly the increase in the extent of spawning (brought about by favourable temperatures) that is responsible for the satisfactory results in warm summers.

Other authors sometimes exaggerate in the opposite direction by denying all correlation between extent and periodicity of spawning and the intensity of setting. Thus STAFFORD (1913) stated: "Spatfall is the all-important event. The value of the oyster harvest does not depend upon the number of eggs spawned, nor upon the number of larvae in the water, but upon the number of successful spat."

Miss CLARK (1935) came nearer to the truth. She observed that variations in the setting of *Ostrea virginica* are often produced by variations in the percentage of the larvae that reach maturity. Although her experiments in vitro showed her that low water-temperatures bring about a protraction of the pelagic period, she failed to see that it is the protraction itself which is the cause that but few larvae reach maturity at low water-temperatures. She stated that the variations in temperature and salinity, record-ed in the field, do not reach values at which they become injurious to oysterlarvae by causing untimely death. It is, however, the protraction of the pelagic period itself that is the dangerous factor, as this exposes the larvae for a longer time to the many dangers besetting them.

Both SPÄRCK (1929) and HAGMEIER (1931) declare that low water-temperatures, by protracting the pelagic stage, will often decrease the percentage of larvae reaching maturity consider-ably.

From my diagrams I shall discuss separately the course of events in the Oosterschelde in the years 1935, 1936, 1937, 1938 and 1939.

1935 [1]) (fig. 21).

The number of adult oyster was rather small in 1935. There-fore the total number of larvae produced in the course of the season of reproduction was not very great. Swarming showed a marked maximum from 10 to 12 July. Just then the water-temperature happened to be very favourable (above 22° C), so that a high percentage of these larvae reached the full-grown stage, which resulted in a marked maximum of setting in the period from 17 to 20 July. Further maxima of swarming of some importance were not observed in 1935. The intensity of

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[1]) In 1935 the larvae were counted by Dr. HAVINGA. Dr. GRIJNS deter-mined the periodicity of setting in 1935 and 1936. The data visualized in the diagram for 1935 (fig. 20) concern the station Strijen.
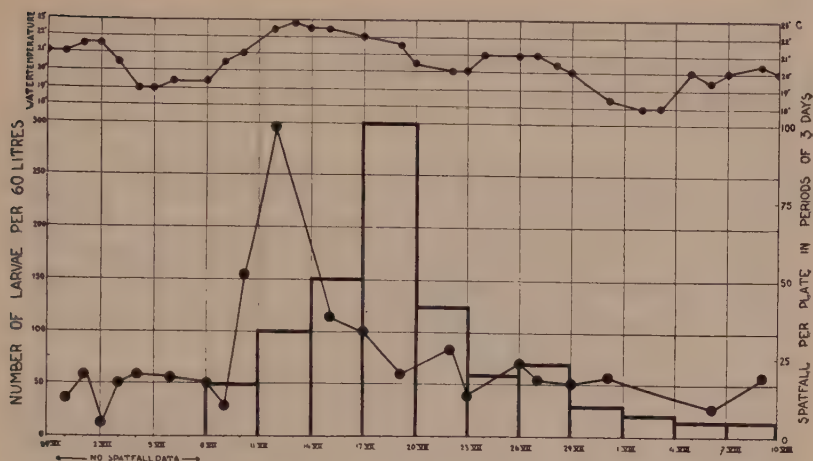
Fig. 21.   Larvae and spatfall. Strijen 1935.

setting in the remainder of the season was of no importance either. 1935 is an instructive instance of a season with a relatively small production of larvae and which yet yielded a good spatfall, which may be put down to the extremely favourable conditions during pelagic life. Most oysterfarmers had placed their tiles just before the maximum of setting occurred, which led to a set of commercial magnitude.

1936 (fig. 7).

From the diagram it can be deduced that the production of larvae was smaller in 1936 than in 1935. The production of larvae in 1936 was unsatisfactory, which may be ascribed to the relatively small number of adult oysters (14 500 000) and possibly to less favourable temperature-conditions in June as compared with June 1935 (fig. 4, table 1). It may be seen from the diagram on temperature-conditions in the summer seasons (fig. 4) that the water-temperature was not nearly so favourable in 1936 as in 1935. The percentage of the larvae that reached maturity in 1936 consequently fell far short of that in 1935. A comparison of the diagram for 1936 with the diagrams on the data obtained in later years shows that the percentage of larvae which reached the full-grown stage in 1936 was about "normal". The total number of larvae and consequently the number of full-grown larvae was quite unsatisfactory, however. In 1936 swarming was moreover not concentrated into a few

days, so that the spatfall was spread over quite a long period. The combination of all these factors resulted in a complete failure of the 1936 set.

1937 (fig. 8).

Since 1937 plankton-sampling has been carried out at the stations Yersche Bank and Kattendijke. It is, however, possible to compare the production of larvae in 1937 with that of 1936, as one of the diagrams for 1936 also refers to Kattendijke. The spatfall may be compared without more ado, as the spatfall-stations Kattendijke and Strijen were used in each season in the course of the investigations.

The diagram for the year 1937 (fig. 8) clearly shows that the production of larvae was greater in that year than in 1936 and 1935, which may be ascribed to the increased number of adult oysters (24 000 000) and possibly to a more favourable tempera-ture in June (fig. 4).

Although temperature-conditions were somewhat more favour-able in 1937 than in 1936, they were not nearly so favourable as in 1935 (fig. 4, table 1).

A clearly marked liberation occurred from 3 to 5 July. Unfortunately the water-temperature was dropping at that time. In consequence of this the pelagic phase was protracted and the percentage of larvae that reached the full-grown stage was rather small. The water-temperature was somewhat higher in the second part of July, which resulted in a higher percentage of larvae that reached maturity. As a consequence of this setting was most intense in the second part of July. Most oyster-farmers, however, had immersed their tiles in the first days of July in the hope of catching spat from the larvae that swarmed in the first days of that month. Adverse weather-conditions interfered, however, while at the time that the spatfall prospects ameliorated (in the second part of July) clean tiles were rather scarce. This was the cause that the practical spatfall, i.e. the harvest obtained by the oyster farmers, was less satisfactory in 1937 than in 1935, although the potential spatfall in the course of the entire season of reproduction did not differ much from that of 1935.

1938 (fig. 9).

Very instructive are the results that were obtained in 1938. The first thing that strikes us in the diagram is that the total

number of larvae produced in 1938 exceeded that produced in 1937, probably owing to a further increase in the number of adult oysters (30 000 000). The swarming observed on 23 and 24 June and on 27 and 28 June was of little importance. This led to small setting maxima in the periods from 3 to 5 July and 9 to 11 July. These numbers of larvae and spat were far too small to be of practical importance.

Heavy swarming occurred early in July, at which time enormous numbers of larvae were liberated. These larvae met with extremely unfavourable temperature-conditions, owing to a spell of adverse weather. The temperature of the water remained below 17° C. during the greater part of their pelagic life. Consequently the pelagic stage was protracted considerably, so that only a very small percentage of all these larvae reached the full-grown stage. This was the cause that the spatfall produced by the larvae liberated early in July turned out a complete failure. Dutch oysterfarmers used to immerse their collectors in the first part of July. When they learned that the production of larvae was great early in July, most of them immersed their tiles in spite of unfavourable temperature-conditions and in spite of our prediction that no satisfactory spatfall was likely to occur in the first part of July. The result was that the practical spatfall was a failure in 1938, especially at Kattendijke.

The occurrence of a fairly large percentage of oysters carrying larvae in the second part of July indicated that another liberation of some importance could be expected. This prediction proved to be correct. The number of larvae liberated towards the end of July and in the first days of August was rather large, though not so large as it had been during the swarming early in July. The water-temperature increased rapidly early in August and soon temperatures of 23° C were recorded. The larvae liberated just then consequently met with extremely favourable temperature-conditions, so that the percentage of those that reached the full-grown stage was uncommonly high, which resulted in an unprecedented intensity of setting. I caught about 300 spat per plate during two successive periods at the station Yersche Bank!

Only an insignificant percentage of this large number of full-grown larvae (fig. 9) succeeded in finding suitable collector material. Clean tiles were very scarce, as most of the tiles had been immersed long before. The few oysterfarmers that placed

some tiles early in August caught an incredible number of spat on them.

So the season of 1938 was characterized by a complete failure in the setting of the larvae of the first considerable maximum of swarming, owing to extremely low water-temperatures in the first half of July, and by an uncommonly intense setting in the first part of August, owing to swarming under extremely favourable temperature conditions. The partical set was a failure in 1938, as but few oysterfarmers had profited by this intense setting. The others had not dared to wait long enough with the placing of their collectors, as we could not guarantee beforehand a spell of extremely fine weather in August to favour the larvae of the second important wave of swarming.

1939 (fig. 10).

The season of reproduction of the year 1939 was characterised by an equable water-temperature. Till the middle of July the water-temperature was about 18° C, after that about 19° C for quite a long period. These temperatures are not very favourable for the development of oysterlarvae The total number of larvae produced in 1939 was still larger than that produced in 1938, which may be ascribed to a further increase of the number of adult oysters (36 000 000).

The numbers of larvae that were liberated towards the end of June and in the first half of July were uncommonly large. The percentage of these larvae that reached the full-grown stage was rather small, however, owing to the rather low water-temperatures.

The larvae liberated in July met with a slightly higher water-temperature than those liberated in June. The setting showed a diffuse maximum in the middle of July, from which it may be deduced that of the larvae produced in July a somewhat greater percentage reached maturity than of those liberated in June. I ascribe this to the small difference in water-temperature mentioned above. The production of larvae early in August led to a small maximum of setting in the second part of August.

Although the maximum of setting in the middle of July was not very large (it was even insignificant in comparison with the setting in August 1938), it yet resulted in a satisfactory practical spatfall, as most of the tiles were in a clean state at the time this setting took place. Several oysterfarmers obtained

harvests amounting to about 50 spat per tile (counted in autumn). On many tiles exposed during the most favourable part of the season the number of spat even exceeded the figure 50.[1])

So the season of 1939 was characterized by an uncommonly large production of larvae, of which only a rather small percentage reached the full-grown stage owing to rather low water-temperatures. But as the intensity of setting was greatest in the middle of July, the oysterfarmers succeeded in obtaining a satisfactory spatfall. The practical set is comparable to that obtained in 1935. It should be remembered, however, that the number of larvae produced in 1935 was but a fraction of that produced in 1939.

Summarizing, it may be stated that the intensity of setting is not always commensurate with the production of larvae. The results obtained in the year 1935 clearly demonstrate that even a small number of larvae may yield a spatfall of commercial magnitude, if only the larvae are liberated within a short period and provided liberation is accompanied by extremely favourable temperature conditions.

The larger the number of larvae, however, the greater the chance that at least part of them will meet with favourable temperature conditions and thus lead to a successful spatfall. The results obtained in 1939 demonstrate that although the percentage of larvae which reaches maturity may be small, owing to rather low water-temperatures, the practical spat-fall may be satisfactory, if only the total number of larvae produced is large enough. The greater the number of adult oysters, the greater the chances of a satisfactory spatfall will be.

Immersion of collectors at water-temperatures continuing below 18° C proved to be ineffective, as in consequence of such low temperatures the percentage of larvae reaching maturity is too small anyway to render a satisfactory spatfall possible.

From what I have said, it will be clear that I do not agree with those authors who believe that setting is commensurate with spawning, nor with those who reject all correlation between the periodicity and extent of swarming and of setting.

The annual extent of the potential spatfall determined with our methods was (number of spat per plate):

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[1]) Owing to the severe winter 1939–1940 by far the greater part of the spat on the tiles died, alas.

Seasons	Stations				
	Katten-dijke	Wemel-dinge	Strijen	Yersche bank	Bergsche bank
1935 8 VII– 3 IX	361		357		
1936 24 VI–17 VIII	39	111	56	180	49
1937 16 VI– 2 IX	272	342	175	447	86
1938 24 VI–29 VIII	357	697	383	1053	171
1939 23 VI–31 VIII	385	433	351	629	109

From these figures it appeared that the potential spatfall was most intense in the year 1938 and that the setting in 1936 was a failure indeed. The most satisfactory harvests were obtained by the oysterfarmers in the years 1935 and 1939.

The reason why the practical set was not very satisfactory in the season of 1937 and was even a failure in 1938, in which year the potential spatfall was greater than in any of the other years under consideration, has been discussed above.

From the foregoing it may be concluded that the water-temperature is a very important factor in the reproduction of *Ostrea edulis*. Although it has been shown that data on the extent of the practical set do not always enable us to determine the extent of the potential setting, we will yet briefly discuss the results obtained by the farmers in the years from 1921 till 1935.

I do not have at my disposal any reliable data on the number of adult oysters that were present in the basin of the Ooster-schelde in these years. Some data on the extent of the spatfall with other information on oyster-culture in the Zeeland Streams in the years since 1921 can be found in Jaarverslagen omtrent den toestand der Visscherij op de Zeeuwsche Stroomen, issued by the Fishery Board of the Zeeland Streams at Middel-burg [1]). Some of these data have been collected in table IV. This table gives a brief survey of the course of events in the years since 1921.

In a special diagram (fig. 4) I have visualized the temperature of the water in the basin of the Oosterschelde in the summers since 1921.

I should like to discuss the years from 1921 to 1931 first.

[1]) The annual report of 1928 was published in Verslagen en Mededeelingen van de Afdeeling Visscherijen Nr. 13 's Gravenhage.

TABLE IV

Years	Numbers of tiles (rounded off)	m³ of shells (rounded off)	Practical set	Remarks
1921	120 000	6 000	satisfactory	mortality
1922	185 000	15 000	complete failure	
1923	300 000	14 000	moderate	
1924	300 000	22 000	moderate	
1925	140 000	47 000	good	
1926	100 000	46 000	very good	
1927	100 000	46 000	good	
1928	100 000	48 000	very good	
1929	70 000	50 000	extremely good	
1930	40 000	40 000	good	
1931	45 000	50 000	moderate	mortality
1932	45 000	33 000	good	mortality
1933	40 000	31 000	moderate	slippers become abundant
1934	350 000	14 000	failure	mortality
1935	1 000 000	2 000	good	
1936	2 000 000	– –	failure	
1937	3 300 000	– –	moderate	
1938	5 000 000	– –	failure	potential spatfall good!
1939	6 000 000	4 000	good	

During these years the greater part of the spat was caught on shell-collectors, mainly old shells of *Cardium edule*. Profuse spatfalls occurred in the years 1929, 1928 and 1926, all of which years were characterized by high water-temperatures during the summer-months (fig. 4).

A complete failure was recorded in the year 1922, in which year the water-temperatures in the summer were extremely low. Moderate spatfalls occurred in 1923, 1924 and 1931, years with moderate water-temperatures in the season of reproduction. In 1923 the month of June was uncommonly cold. Although a considerable mortality was recorded in the year 1921, which probably was the cause of a decrease in the production of larvae, the spatfall was nevertheless satisfactory then, probably owing to the favourable temperature conditions.

In the following years other factors interfered. The first slippers (*Crepidula fornicata*) were observed in the Zeeland Streams in 1929. An uncommonly rapid propagation of the slipper-limpet soon made it impossible to continue with the sowing-out of

Cardium-shells, as the oyster-spat got overcrowded by slippers. The volume of shells sown out decreased rapidly in the years following 1933, while commensurately the number of tile-collectors increased from year to year.

Moreover a considerable mortality was caused by shell-disease in the years following 1930 (HAVINGA 1931, KORRINGA 1939) and most of the surviving oysters were in a bad condition. In spite of this the spatfall in 1932 was quite good, no doubt owing to the very favourable temperature conditions in the summer of that year.

The results obtained in the last five years have already been discussed above. It may be seen in the diagram (fig. 4) that temperature conditions were far more favourable in 1935 than in the years following, which may partly account for the satis-factory setting by which this year, notwithstanding the small number of larvae, was characterized.

Summarizing, I believe that the data on the years from 1921 to 1934 clearly show that my conclusion (based on the data from 1935 to 1939) that water-temperature is a very im-portant factor in the propagation of *Ostrea edulis*, is correct. If no other factors interfere and provided there is a large number of healthy adult oysters, we may expect warm summers to be productive of profuse potential spatfals and cold summers to result in partial or even complete failures. This may be ascribed to the influence of temperature on the duration of the pelagic stage and thus on the percentage of larvae reaching maturity. Moreover, favourable temperature conditions may incraese the production of larvae, as has been discussed above.

In the next section it will be discussed how science may help the oyster farmers to obtain a satisfactory practical set, whenever the potential setting in good.

XXVI. THE PREDICTION OF THE SPATFALL

> "Thousands of dollars would be saved annually by the oystermen if they would determine with any approximate accuracy the data when attachment of the young oysters would occur." (WINSLOW, 1884).

The oystergrowers are faced with the problem of deciding when to plant cultch, so that it shall not be silted over or covered with organic growth before the larvae are able to attach. In

many cases the cultch is laid out far too early, with the result that the maximum catch of spat is not obtained. Sometimes collectors are immersed when the potential maximum of setting is over, which does not increase the chances of obtaining a satisfactory practical set either. In the years before 1935 Dutch oysterfarmers in deciding when to plant cultch had to consider the dates on which the collectors were ready to be planted and had to trust to the experience gained in former years, but most of all to their good luck. The desire for more reliable indications induced several oysterfarmers to plant "test-collectors". Small numbers of tiles were planted at intervals and as soon as these tiles were found to be covered with spat, the farmer started to plant his entire supply of collectors. As the spat can only be seen with the naked eye about a week after attachment, it goes without saying that this method frequently led to disappointments, the best time for setting often being over when the cultch was planted.

The first attempt to predict the spatfall was made by JULIUS NELSON (1907). He examined plankton-samples and tried to predict the probable date of setting from the stage of development of the larvae. The simultaneous spawning, discussed above, accounts for the fact that often all the pelagic larvae of *Ostrea virginica* show the same stage of development, which makes it easy to predict the date of setting with reasonable accuracy. THURLOW C. NELSON (1917) states that it is possible to predict the time of setting within two days by means of the technique developed by him.

It is not only necessary to predict the date of setting, but also the intensity of the spatfall. The latter problem is more difficult, as the percentage of the larvae reaching maturity is largely dependent on environmental conditions. The solution of this problem requires extensive quantitative observations.

In the main centres of spat-production in France, le Morbihan and the basin of Arcachon, oysterfarmers have for several years been assisted in choosing the right moment for cultchplanting. Each summer-season the French investigators issue bulletins in which they publish the number of larvae counted in their plankton-samples, the water-temperatures recorded and often the "fixation-coefficient". It has been discussed above why I refuse to believe in the reliability of the "fixation-coefficient". Moreover the time elapsing between the observation of a favour-

able "fixation-coefficient" and the occurrence of the spatfall is rather short. I am of opinion that with the Dutch methods and frequency of sampling the dates and intensity of setting can be predicted with a higher degree of accuracy than with the French. It should be remembered that *Ostrea edulis* does not show simultaneous spawning, so that the prediction of the spatfall of *Ostrea edulis* presents greater difficulties than that of *Ostrea virginica*.

The enforced return to the system of tile-collectors in the Oosterschelde led to the establishment of a system of predicting the spatfall in Holland. Once or twice a week bulletins are issued in which are given the number of larvae per 100 litres of water, its composition in size-classes, the water-temperatures recorded and the setting prospects. We not only predict the dates of setting, but also the intensity of the spatfall. When predicting the setting, I always compare the number of spat that attached under similar conditions in previous seasons. The high frequency of our sampling, the regular determination of the potential spatfall and the division of the larvae in size-classes have proved to be of the greatest use for this purpose.

When a considerable maximum of swarming occurs under very favourable temperature conditions, we advise oysterfarmers to plant soon as many of their collectors as possible. If no swarming occurs or if it occurs under extremely unfavourable temperature conditions, as was the case in the first half of July 1938, I make it known that no setting of commercial magnitude is likely to occur. When moderate swarming takes place under rather favourable conditions or when a considerable swarming occurs under fairly unfavourable conditions, I announce that some spatfall is likely to occur within about 10 days, provided the water-temperature does not drop. As soon as the number of larger larvae is found to be increasing considerably, the setting prospects are announced with greater precision.

When the season is well on its way already and no favourable predictions are forthcoming (as was e.g. the case in 1938), the oysterfarmers get into a difficult position. If they plant their cultch in spite of unfavourable predictions, the odds are that they will obtain an unsatisfactory set. If they wait any longer, the setting prospects may ameliorate, i.e. if the water-temperature and the number of larvae should happen to increase, but it is

also possible that no favourable predictions follow, in which case the chance of obtaining a satisfactory spatfall is still smaller. It is advisable in such cases not to put all one's eggs in one basket. When the larvae of a considerable maximum of swarming have not led to a spatfall of some importance, we proceed to predicting if a subsequent swarming of some importance is likely to occur. For that purpose we examine fairly large samples of adult oysters from different oysterbeds in order to ascertain the percentage of those that is incubating. So far it has been impossible, however, to predict so long before whether a spell of fine weather is likely to occur at the time that this subsequent swarming may be expected.

COLE (1939) does not think it impossible that *Ostrea edulis* is cleverer than our meteorologists: "It is remarkable that during the last three seasons the major liberation of the season has occurred just after the beginning of the first lengthy hot spell, as if the breeding oysters had sensed in some way the indicence of a prolonged spell of fine weather during which the larval development could be completed satisfactory."

Unfortunately Dutch oysters appear to be unable to sense this, for liberation has but too often occurred during adverse weather or during a falling water-temperature.

Many attempts have been made to predict the spatfall at long notice. It is partly the absence of quantitative data on larval development that has compelled investigators to predict the spatfall long before and partly these attempts have been made to meet the wishes of the oyster farmers, who want to be informed as soon as possible of the expectations about the date of the spatfall and the intensity of setting. HOPKINS, ORTON and PRYTHERCH belong to the first category.

HOPKINS concluded (1937) that the periodicity in spawning parallels the periodicity in setting in some respects only. HOPKINS did not follow the development of the pelagic larvae. It is my belief, however, that quantitative observations in the course of the pelagic stage are especially indispensible in species of oysters with such a long pelagic period as *Ostrea lurida* (30 to 40 days). HOPKINS has not provided himself with data from which to predict the intensity of setting and as for the time of setting he states that attachment is likely to show a maximum in the third tidal period following on that during which spawning starts. HOPKINS

often observed maxima in the frequency of setting about the time of the greatest tidal range, but he does not yet know the exact reason for this phenomenon.

PRYTHERCH (1929, 1934 b) does not base his predictions on observations on quality and quantity of pelagic larvae either, although *Ostrea virginica* shows a less complicated composition of larval age-groups in the plankton (owing to simultaneous spawning) than the gradually spawning incubatory species of oysters. PRYTHERCH observed that the local hydrographical conditions are the cause of a rapid warming up of the water during spring-tides, which is in its turn the cause of maxima of spawning at spring-tides. PRYTHERCH bases his predictions of the time of spawning on observations of the water-temperature, for which he uses a long-distance thermograph. It has been stated before that PRYTHERCH neglects the influence of environmental conditions on the percentage of larvae reaching maturity which omission I am inclined to ascribe to a lack of quantitative data on the pelagic larvae. Before spawning begins PRYTHERCH is able to predict its intensity from studies on the thickness of gonad-tissues, which according to him is liable to considerable annual differences, resulting in proportional differences in the spatfall.

It may be seen from HIGGINS' papers (e.g. 1933, 1937) that announcements about the condition of the oyster, changes in water-temperature and the dates at which spawning and setting may be expected are being continued in the district of Long Island Sound.

NELSON (1926, 1928 c) too, predicts the dates at which spawning of *Ostrea virginica* will start, for which he bases himself on observations of the water-temperature. His methods have been described in a previous section. He also predicts the dates of setting, for which he bases himself on quantitative observations of larvae.

As regards the prediction of the spatfall ORTON states (1937): "An examination of samples of oysters on each bed each year is therefore desirable to find out the rate of spawning and from this information the most propitious time for the laying of cultch." "The density of the subsequent fall will be proportional to the percentage of black-sick oysters." "It is suggested that a reasonable course is to lay cultch when about 5% of the stock are black-sick, and there is in addition at least 5% of younger stages

of sickness." As this method requires the sacrifice of a considerable number of adult oysters (ANON. 1928), ORTON suggests (1927 e, 1928 b) a more economical method of testing adult oysters. The oysters can be exposed to the air till they open their shells a little, which enables us to see whether they are incubating. After this they are thrown back into the water. It will be clear from the foregoing that the fitful weatherconditions on the North-sea coast preclude a fixed proportion between the intensity of spawning and the intensity of setting. Consequently it is my opinion that the application of ORTON's methods of prediction cannot lead to reliable results here.

Nevertheless a determination of the percentage of oysters carrying larvae is useful, if we want to know whether subsequent spawning of some importance is still to be expected, in case the larvae of the first maximum of spawning should have failed to yield a commercial spatfall (as, for instance, in the Oosterschelde in 1938).

As regards the prediction of setting at long notice I agree with VOISIN (1931, 1933) who states that, owing to the fitful weather-conditions, such predictions must always be unreliable on the west coast of Europe.

A reliable prediction of setting at short notice, on the contrary, appeared to be very well possible in Holland.

As the Dutch oystermen began to realize that those who followed our advice generally caught the best sets of spat, their interest in the predictions has increased rapidly. With our method we try to synchronize the practical setting with the largest maxima in the potential setting. In this way we try to reduce the enormous difference between the number of mature larvae occurring in the course of the season of reproduction and the number of successful spat on the collectors.

XXVII. THE INFLUENCE OF LIGHT ON THE SETTING PROCESS

The pigment-spots or pallial eyes of mature oysterlarvae have been the subject of considerable controversy. There can, however, be no doubt about the eye-like structure of these organs in the larva of *Ostrea edulis* (ERDMANN 1934, COLE 1938 a). The black colour of these spots is due to heavy pigmentation. The pigment-spots disappear soon after attachment. Phagocytes appeared to play a part in the process of desintegration. COLE (1938 a)

observed phagocytes heavily loaded with pigment-grains leaving the fading pigment-spots in recently-set spat of *Ostrea edulis*.

PRYTHERCH (1924, 1934 a) has not observed any response to changes in light-intensity or colour by the larvae of *Ostrea virginica*. He came to the conclusion that the pigment-spots are not light-sensitive organs at all, but fulfil a quite different function. It is his belief that they are leucocyte-generating tissues. PRYTHERCH probably made the same observation as COLE did and based his conclusion on the fact that shortly after attachment he observed many phagocytes in the proximity of the pigment-spot. COLE's interpretation of this fact is the more plausible, however.

As the eye-like structure of the pigment-spot has been established (at least for *Ostrea edulis*), we should not be surprised to find that light plays some part in the selection of a site for attachment. Only a few investigators made deliberate experiments to investigate this matter, however. Others based their conclusions on accidental field-observations. Thus PETERSEN (1908), FOLPMERS (1924) and ORTON (1937 a) found more surviving spat of *Ostrea edulis* in situations where the light is subdued, for instance, on the shaded sides of collectors. ORTON and PETERSEN admit, however, that this fact does not necessarily indicate that oysterlarvae prefer dark places for attachment. For it is possible that a growth of plants, macroscopical or microscopical, on the light side of the collectors will soon hinder attachment, while the shaded sides may remain longer in a condition suitable for attachment. Moreover it is possible that the death-rate of the spat on the two sides of the collectors is different, for the light sides are as a rule also more exposed to enemies and to silting. To eliminate such factors it is necessary to expose test-collectors for short periods and to count the spat a few days after attachment.

PRYTHERCH (1924) states that the setting of the larvae of *Ostrea virginica* occurred as heavily on the light sides of the collectors as it did on the darkened sides, it being assumed that both sides were equally clean. In a later paper (1934) he tells us that during eight years of experimentation he did not observe any influence of light on the setting process. He does not describe, however, what kind of experiments he made to investigate this matter.

NELSON (1926), on the other hand, states that the pigment-

spot of *Ostrea virginica*, "although unable to form an image like a true eye, is sensitive to light." "In the presence of light the eyed larvae of the oyster are stimulated and continue moving until they get into a shaded place where they become quiescent."

Consequently the shaded under-surfaces of the collectors are supposed to catch more spat than the upper-surfaces during daylight. It is my belief that it was the observation of a heavier fall of spat on under-surfaces that induced NELSON to form his hypothesis of a stimulated swimming-activity in illuminated places. Whenever he found more spat on the undersides of collectors (1927, 1934), he used this hypothesis in explanation of this phenomenon. NELSON neglects, however, the influence of the angle of surface on the intensity of setting, so that we are not justified in concluding from his observations that light plays a part in the setting process.

Deliberate experiments to investigate the influence of light on the setting process have been carried out by YOKOTA (*Ostrea gigas*), HOPKINS (*Ostrea lurida*) and COLE and KNIGHT JONES (*Ostrea edulis*). YOKOTA (1936) placed full-grown larvae of *Ostrea gigas* in a glass filled with seawater and screened off half the glass (source of light: electric lamp 100 volt, 60 watt, 36 cm above the water). The distribution of the spat on the bottom of the glass appeared to be almost uniform in spite of the great difference in illumination. YOKOTA concludes from these results that at the time of setting *Ostrea gigas* does not react negatively to light.

HOPKINS (1935, 1937) placed wire frames containing plates of clear glass on an oysterground, so that the plates were held in a horizontal position. The upper-surfaces of the plates of one set were painted black, the others were left clear. He found that the numbers of larvae setting on the under-surfaces of clear and darkened glass were more or less the same, which shows that under field-conditions the larvae of *Ostrea lurida* do not select shady places for attachment. HOPKINS concludes that light is not an orienting factor in the setting behaviour of this species.

COLE and KNIGHT JONES (1939) used the same device in the tanks that are in use for spat-production in England (*Ostrea edulis*). They observed a marked preference on the part of the larvae for the undersides of the dark plates (in 18 sets: under-surfaces of clear glass 228 spat, under-surfaces of dark plates 735 spat). This selection of shady places appeared to

be confined to the daylight hours, while plates exposed at night showed no difference in the number of spat caught on the undersides of the plates. They concluded that during daylight the larvae of *Ostrea edulis* undoubtedly tend to select a shady situation for attachment.

In the same season that COLE and KNIGHT JONES made their observations in tanks (1938), I used HOPKINS' device in the Oosterschelde. Experience had previously taught me that plates of smooth glass exposed in the field are far less suitable to catch spat of *Ostrea edulis* than plates of various other materials. This phenomenon will be discussed more amply in one of the following sections. Therefore I used ground glass for this experiment, this being a little more suitable for attachment and yet pervious to light. The plates were placed horizontally in containers of reinforced concrete. Clear plates and plates with a black coating on their upper surfaces were exposed together in one container. In the field ground glass catches somewhat more spat than smooth glass, but the number of spat counted on the undersides of the plates appeared to be too small to base conclusions on, for, owing to the influence of the angle of surface on the intensity of setting, the undersides of the plates caught far less spat than the uppersides, as will be discussed presently. The total catch of the undersides of 3 series of 3 plates, each series exposed during 6 days, was:

<div align="center">
upper-surfaces clear: 11 spat
upper-surfaces black: 43 spat
</div>

There are, however, many data available on other series of spatfall experiments in the Oosterschelde, which might show that light influences the setting process. In the containers I often placed three coated plates in a horizontal position, the one right above the other with an interspace of 3 cm. Consequently the uppersides of the two lower plates caught far less light than the uppersides of the topmost plates. The numbers of spat I counted on the uppersides of the plates of these series are recorded in the table on page 191.

These data do not allow me to conclude that under field-conditions the larvae of *Ostrea edulis* tend to select shady situations for attachment.

In the summer of 1938 I shaded one half of a group of tile-collectors, seeing to it that factors other than light did not change

Series	Topmost plate	Second plate	Third plate
4 series July 1938	191	161	206
3–12 July 1938	69	30	40
12–21 July 1938	53	23	36
2–8 August 1938	224	–	70
8–14 August 1938	1194	–	1031
8 series 1939	903	744	723
8 series 1939	760	476	504

under the cover. In September I counted about an equal number of spat on the tiles under the cover and on the tiles that had been exposed to the sunrays.

As the poor spatfall on smooth glass and ground glass prevented me from obtaining reliable data on the influence of light on the setting process in the field with HOPKINS' device, I constructed another apparatus to investigate this matter. The factor light alone had to be changed, while the other factors, especially the accessibility for the water and with it for the oysterlarvae, had to remain constant. I used one of my large containers, in which there is room for 12 plates. I numbered the places in the container (fig. 22) and I made a sluice of clear glass in places 1 and 2,
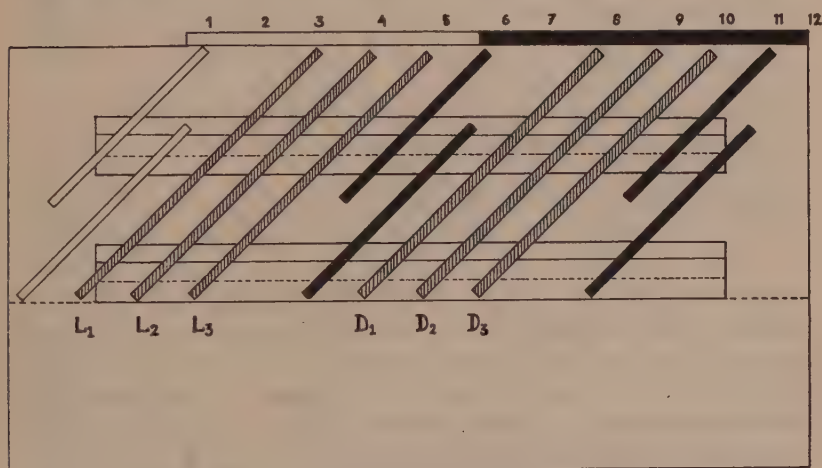
Figure 22. Container with light and dark compartment.
Longitudinal section.

put test-plates in places 3, 4 and 5, making a sluice of glass coated with black paint (but for the rest similar to the first

sluice) in places 6 and 7, putting test-plates in places 8, 9 and 10 and making another dark sluice in places 11 and 12. The container was covered with a glass plate of which one half was coated with black paint. I did not shut the long narrow apertures in the sidewalls of the container (cf. fig. 19), but I constructed sluices of zinc in them, so that no lightrays could penetrate into the inside of the container through these apertures while the larvae-bearing water could flow in freely through them.

  In this way the container was divided in two compartments with test-plates. In one compartment the light could enter freely and in the other complete darkness prevailed, while the larvae-bearing water could enter both compartments equally well. Moreover I regularly interchanged the places of the dark and the light compartment in the container. To this end the plates of the sluices and the covering glass plate were made to fit in brass rails, so that they could easily be exchanged. The results obtained with this apparatus are the following:

Series	L. I	L. II	L. III	L. Av	D. I	D. II	D. III	D. Av.
2 VII– 8 VII 1939	38	21	22	27	43	13	16	24
11 VII–14 VII 1939	9	18	22	16	17	14	10	14
14 VII–20 VII 1939	79	46	37	54	47	30	39	39
20 VII–26 VII 1939	36	20	15	24	29	16	22	22
26 VII– 4 VIII 1939	60	47	31	46	34	43	56	44
4 VIII–10 VIII 1939	30	27	22	26	65	26	16	36
10 VIII–16 VIII 1939	19	13	38	23	26	19	16	20
16 VIII–22 VIII 1939	31	29	32	31	49	38	28	38
Total	302	221	219	247	310	199	203	237

  No more spat was caught in the dark compartment than in the light compartment. So far my field-observations have not demonstrated that light plays an important part in the setting process of *Ostrea edulis*. This is in agreement with HOPKINS' field-observations on *Ostrea lurida*, but not with the results obtained by COLE and KNIGHT JONES.

  Although light appears to be no orienting factor in the setting behaviour of *Ostrea edulis* under field conditions, it is not impossible that light influences the setting process, in cases when the intensity of the light exceeds a certain degree. The water in the Oosterschelde is rather turbid and above the containers

we find from 2 to 6 metres of water, so that the intensity of light on the bottom, on which the containers are placed, is not very great.

COLE placed the plates in his tanks about one metre below the surface and as the water in the tanks was moreover very clear and completely free from silt, his plates received far more light than HOPKINS' and mine. This problem calls for further experiments under controlled laboratory conditions. YOKOTA's laboratory experiments have not yet been carried out on the larva of *Ostrea edulis*.

Summarizing, I conclude that light evidently does not play any part in the setting process of *Ostrea edulis* under field-conditions in the Oosterschelde, but that COLE's experiments in tanks point to the possibility that oysterlarvae tend to select shady situations for attachment, when the intensity of the light or the difference between "light" and "dark" is great enough. It is doubtful, however, whether this influence is great enough to play any part in setting under field-conditions, especially when the water is rather turbid and the range of the tide is great, as is the case in the Oosterschelde. It is not probable that light-perception by the pigment-spot is indispensible in the setting process of *Ostrea edulis*.

The comparison of the spatfall during day and night is closely bound up with the problem of the influence of light on the setting process.

If NELSON's hypothesis (1926) of a stimulated swimming activity in the presence of light is correct, we may expect a greater intensity of setting during darkness than in daylight. NELSON did not investigate this matter, however. HOPKINS (1937) demonstrated that the setting of *Ostrea lurida* often proceeds equally well at night as during the day. COLE and KNIGHT JONES (1939) compared the setting in tanks during 7 hours of darkness with that obtained during a period of daylight of the same length. Their figures show a marked tendency of the larvae to attach themselves during daylight hours, for the average number of larvae setting per daylight period was about three times that which set during a period of darkness of the same length. They do not explain how this phenomenon is to be reconciled to their conclusion that the larvae of *Ostrea edulis* tend to select shady situations for attachment. Is it perhaps the

contrast between light and darkness that creates favourable setting conditions? Or is it possible to interpret the selection of shady places in the tanks as a tendency to avoid too intense an illumination and can the difference in setting during night and day be correlated with differences in the swimming behaviour of the mature larvae?

I placed 3, sometimes 6 plates in a container at the station Yersche Bank during dark night-hours. As I knew beforehand that the setting is not intense enough to obtain an adequate number of spat in one night, I placed the plates in a zinc container filled with filtered sea-water during the day and put the plates back in the concrete container on the sea-bottom for the next night. Thus the plates were exposed for three consecutive nights. I placed the daylight set in the same container at the same station for a period of the same length. To eliminate the influence of the tidal cycle on the intensity of setting, I placed the daylight set in the container during exactly the same part of the tidal cycle as the night set. Some series caught too little spat. One of the series (i.e. 8 VIII-11 VIII 1938) caught a great number of spat, as it had been exposed during the most favourable part of the setting season and as on that occasion I used a container in which the plates were held in a horizontal position.

Period	Exposure		Spatfall			
	Night-Set	Daylight-Set	Night-Set	Av.	Daylight-set	A
8 VIII–11 VIII 1938	20.30–5.30	8.30–17.30	82–61–75 60–78–68	71	200–362–356 332–362–304	3
11 VII–14 VII 1939	22.00–5.00	10.00–17.00	1–2–3	2	3–4–2	
16 VIII–19 VIII 1939	21.00–6.00	9.00–18.00	5–4–4 0–7—	4	18–18–5 15–17–2	1

The above results seem to confirm the conclusion of COLE and KNIGHT JONES that the setting of *Ostrea edulis* is more intense during daylight than during the night.

I cannot yet give any plausible explanation of this phenomenon. I have never observed a marked difference in the vertical distribution of full-grown larvae during night and day

(fig. 11). Other conditions, such as current-velocity, salinity and water-temperature, are practically the same during night and day. Only the factor illumination differs. However, I did not catch more spat in the light than in the dark compartment of the apparatus with which I investigated the influence of light on the setting process. This apparatus was placed at the same station and at an equal depth as the container with the plates in the experiments described above.

Consequently I do not yet see how the difference in illumination can bring about a difference in setting between night and day.

I have remarked in a preceding section that full-grown larvae sometimes seem to show a slight difference in their vertical distribution during night and day (fig. 11), but that the numbers of mature larvae I counted in my samples were too small to base conclusions on. Perhaps it is this factor that holds the key to the problem.

COLE and KNIGHT JONES suppose that the difference between the setting intensity during night and day is possibly due to a decrease in the swimming activities of the larvae at night, resulting in a general sinking towards the bottom (which has not been observed by them, however), but my investigations clearly demonstrate that such a general sinking towards the bottom during the night most certainly does not occur in the Oosterschelde (fig. 11, 12).

XXVIII. THE INFLUENCE OF DIRECTION AND VELOCITY OF THE CURRENTS ON THE SETTING PROCESS

We can distinguish a favourable and an unfavourable influence of the currents on the setting process. In the first case we will consider how the currents continuously supply full-grown larvae to the places where the collectors have been planted. The larvae-bearing water flows along and through the masses of cultch material and provides them with the required larvae. Only when the cultch has been planted in too compact masses, we shall observe that the supply of larvae has been smaller in the centre of the masses than in the outer zones. Thus it has often been stated that as a rule less spat is found per shell in the centre of the wire bags filled with shells, which are in use in America [1]).

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[1]) The same experience has been gained in the Oosterschelde with wire-covered trays filled with shells.

BONNOT (1937 b) also mentions a less intense setting of *Ostrea lurida* in the centre of his batteries of wooden collectors, which resemble the "plateaux collecteurs" of the French oystermen. It is an important problem in the production of seed-oysters to find adequate methods to utilize the natural supply of larvae to the best advantage. The same quantity of cultch can be supplied in different ways. The best method is that which makes it easy for the larvae to reach the surfaces. It has been discussed in a preceding section that many collectors may be planted successfully in one place, owing to the enormous difference between the number of full-grown larvae and the number of successful spat. Accumulation of collectors may yield good results, provided the larvae-bearing water can flow freely through the cultch masses. Generally speaking, we may say that the more easily the larvae can reach the surface, the more spat will be caught. Thus COE and ALLAN (1937) tell us that more spat of *Ostrea lurida* settled on the outer surfaces of their experimental collectors than on the other surfaces.

HOPKINS (1935, 1937) placed glass plates parallel and perpendicular to the direction of the flow of the tide. The plates were placed either vertically or at an angle of 45°. In either case the plates that had been exposed parallel to the current definitely caught more spat (*Ostrea lurida*), presumably because more larvae-bearing water comes into contact with the surfaces of these plates. HOPKINS' conclusion that setting may be proportional to the rate of current is not justifiable, however, for the currents may just as well have an unfavourable influence on the setting, especially when the current is strong, as will be discussed below.

SCHAEFER (1937) obtained the same results with *Ostrea gigas*, using the same device as HOPKINS.

Independently of these experiments I tried to find out whether the direction of the current has any influence on the setting of *Ostrea edulis* in the Oosterschelde. Here it is impossible to catch an adequate number of spat during the short period of flood or ebb. I constructed an apparatus that holds the plates in the desired position for several days. An iron axis was erected on a solid foot of reinforced concrete, which could be placed on the sea-bottom (fig. 23). A structure of wood and iron, provided with a zinc rudder, could revolve freely round the axis. Brass frames were attached vertically to this construction, either parallel with the rudder or perpendicular to it. In these frames

the coated glass plates were held. A rope and a buoy were attached to the iron ring at the top of the axis in order to make it possible to find back the apparatus in the field.

The results obtained with this apparatus are the following:

Series	Parallel to the current				Perpendicular to the current			
5 VIII–11 VIII 1938	40	17	36	40	19	16	25	22
11 VIII–23 VIII 1938	21	20	30	15	13	18	11	11

Other series caught less spat.

Although these numbers are not very great, which is mainly due to influence of the angle of surface (vertical plates catch far less spat than those held at other angles), they suffice to show that the plates placed parallel to the direction of the current catch more spat than those perpendicular to the tide. This is in accordance with the results obtained by HOPKINS and SCHAEFER with other kinds of oysters.

However, the currents may also have an unfavourable influence on the setting process. YOKOTA (1936) observed the setting behaviour of *Ostrea gigas* in vitro. When by means of a syringe he produced currents in his vessel, the mature larvae often appeared to be able to continue crawling on the substratum, as the body was supported by byssus-threads. When the current was strengthened, the byssus snapped off, however. Then the mature larva could only start crawling again after the current-velocity had been sufficiently decreased. From this we see that when the current is too strong the larvae are washed off from the substratum and that attachment will be impossible for the greater part of the tidal cycle in places where strong currents prevail.

NELSON's view (1921), though not based on such experiments, is in accordance with the observations of YOKOTA. NELSON states that the setting process of *Ostrea virginica* "cannot be carried out successfully except in relatively quiet water, hence in places where swift currents prevail, the larvae attach during the period of slack water between tides, or they creep in between shells or other objects, where the current is practically nil."

PRYTHERCH's observation (1929) that the larvae of *Ostrea virginica* mainly attach themselves to the leeward sides of the collectors also accords with these data.

Figure 23. Apparatus for investigating the influence of the current
on the setting process. (Photo Havinga).

In investigating this matter I used the apparatus described
above (fig. 23). I changed, however, the position of the brass
frames. All the frames were placed horizontally. Four of the
eight frames were sheltered from the current in front and at
the sides with zinc screens. The other frames remained exposed
to the currents as fully as possible.

For the results obtained with this apparatus see page 199 top.
The fully exposed plates apparently caught less spat (16 per

Period	Small shelter		Large shelter		Fully exposed			
5 VII–11 VII 1939	18	29	43	37	11	14	9	6
11 VII–17 VII 1939	55	61	81	73	19	19	16	10
17 VII–23 VII 1939	–	13	38	36	19	–	6	9
23 VII–29 VII 1939	19	–	19	36	7	18	8	1
29 VII– 7 VIII 1939	27	–	60	22	14	17	9	–
7 VIII–13 VIII 1939	18	13	19	–	18	12	6	–
13 VIII–19 VIII 1939	28	33	69	75	29	21	34	–
19 VIII–25 VIII 1939	60	–	48	36	43	42	21	–
On an average	31		45		16			

plate on an average) than the sheltered ones (31 and 45 per plate on an average). The plates that were exposed behind the larger shelters showed an equal distribution of the spat over their entire surface. The majority of the spat on the plates behind the smaller shelters, however, appeared to have congregated on that side of the plate that had been close to the zinc shelter. The side farthest away from the screen was apparently not sufficiently sheltered, which accounts for the smaller number of spat on these plates.

The current-velocity at the station where I placed this apparatus amounts from 30 to 50 cm/sec. for the greater part of the tidal cycle.

My data clearly indicate that in the Oosterschelde the spatfall is more intense in sheltered places. I dare not decide whether all the spat on the fully exposed plates attached during or about slack water, but I am inclined to think so.

From the foregoing I conclude that strong currents hinder the spatfall considerably, because the larvae are washed off from the substratum by them. In places where the tide is rather swift, attachment is probably only possible during slack water and during the tide only in those parts of the collectors that are sufficiently sheltered from the currents.

If the currents are slow their favourable effect (i.e. the continual supply of larvae) will preponderate, but if the currents are strong, the unfavourable effect (i.e. the washing off from the substratum) will become operative. HOPKINS' assumption that setting may be proportional to the rate of current only holds good in the case of slow currents.

XXIX. THE SPATFALL IN THE COURSE OF THE TIDAL CYCLE

The distribution of the spatfall over the different stages of the tidal cycle has not often been thoroughly investigated.

In a preceding section I have related already how PRYTHERCH (1929) found that in the Long Island Sound the setting of *Ostrea virginica* occurs mainly roundabout low slack water and during the early flow (fig. 20). PRYTHERCH states that the setting gradually becomes less intensive as the velocity of the currents increases and finally ceases altogether on the current attaining a velocity of 10 cm/sec. As I have stated above (fig. 20) it is my opinion that the main factor causing a concentration of the spatfall during low slack water in the Long Island Sound is the variation in the number of larvae per unit of water during the tidal cycle. It is my belief that other factors, such as the copper-content of the water and the current-velocity, exercised less influence on the intensity of setting in this particular case.

HOPKINS (1937) ascertained in several stations in the Puget Sound the number of spat of *Ostrea lurida*, caught hourly per unit of cultch. He used batteries of glass plates supported in wire frames. He invariably found that the least spat was caught at low tide, when the dikes are exposed and the water-temperature is high. The maxima of setting in different stations did not always occur at corresponding stages of the tide. HOPKINS tried to establish a correlation between the intensity of setting and variations in factors, such as the rate of current, temperature, salinity and pH. He concluded that in some cases there seemed to be a correlation between the rate of current and the frequency of setting. HOPKINS neglected, however, the most important factor, i.e. the variation in the number of full-grown larvae per unit of water!

HOPKINS writes that: "Chance is a large factor in determining whether the water in the particular place happens to contain larvae. For this reason the error involved in the tests is considerable."

I tried to ascertain the intensity of setting in the course of the tidal cycle in the Oosterschelde. I knew beforehand that it is impossible here to obtain adequate numbers of spat on plates exposed during one hour. Even if we divide the tidal cycle in four sections: high water, ebb, low water and flow,

each of three hours' duration, adequate numbers of spat can only be caught when the spatfall is exceptionally intense. Under normal conditions a three hours' period of exposure is too short. Therefore I placed the plates during three hours in the containers on the sea-bottom, replaced them by an other set of plates during the next section of the tide and put the removed plates temporarily in a zinc container, filled with freshly filtered sea-water. In this way I used four sets of three plates. The first three plates were put back in the container when the three other sets had had their turn. I continued exchanging the plates during 3 × 24 hours. As the exchanging of the plates during night and not infrequently under unfavourable weather-conditions is by no means a sinecure, it was not easy to obtain many of such series. The results were rather disappointing. The total number of spat on all of the 12 plates was in most cases insignificant, although the number of spat caught by the plates of the normal series, placed at the same station during the same three days, was rather considerable. The same phenomenon, i.e. the decrease in the total number of spat caught according as the period under consideration is subdivided in an increasing number of shorter periods, has been observed by Hopkins (1937). Hopkins suggests that "those larvae which had not completed the setting process released their hold when the plates were withdrawn from the water, so that possibly only those that began to set soon after the plates were immersed were able to attach permanently."

In the course of my investigations this exchanging of plates during 3 × 24 hours has been carried out five times, viz. four times at the station Yersche Bank and once at the station Katten-dijke. Only one of these five series caught a moderate number of spat, although the total catch of the entire set remained far below the number of spat caught on the ordinary series of plates, which remained constantly in the water during those three days. This was not caused by the death of the spat during the hours spent in the zinc container, for in that case the empty shells of the dead spat could have been detected on the plates. Moreover I obtained excellent results with other series of plates which also remained in the zinc container for a considerable time. Hopkins' explanation is probably correct, but the problem calls for further investigation.

Series exposed from 14 to 17 July at the station Yersche Bank:

	Period of exposure	Number of spat on the plates			Average
Low water	(1½ h. before L.W.–1½ h. after L.W.)	3	8	6	6
Flood	(1½ h. after L.W.–1½ h. before H.W.)	4	4	1	3
High water	(1½ h. before H.W.–1½ h. after H.W.)	16	23	19	19
Ebb	(1½ h. after H.W.–1½ h. before L.W.)	6	7	4	6

These results seem to indicate that at the station Yersche Bank the setting is most intense during high water.

I have tried to find other methods to investigate this matter, as the exchanging of the plates during 3 × 24 hours is no trifle and as such series of plates often bore little spat. In one of the preceding sections I have shown that the great majority of the spat of *Ostrea edulis* attaches with the umbos pointing in about the same direction, provided the substratum is not held in a horizontal position. An analysis of an adequate number of spat enables us to infer which side of the collectors has been uppermost at the time of setting.

If we imagine a collector that changes its position in the course of the tidal cycle, so that it is not always the same side of the collector that is uppermost, we might be able to deduce by analyzing the orientation of the spat which side of the the collector has been uppermost at the time of the greatest intensity of setting. As, alas, the umbos of the spat do not all point precisely in the same direction, we shall need a fairly great number of spat, if we want to use a method of ascertaining the intensity of setting in the course of the tidal cycle, based on the revolving of collectors.

I constructed an apparatus based on the idea explained above (fig. 24). The centre of it was formed by a solid time-piece capable of running for several days. This time-piece was shut up in a water-tight iron box, which was placed on a solid foot of reinforced concrete. Only the axis of the time-piece protrudes from the iron box at an angle of 45° with the horizontal surface. A set of three coated plates could be attached perpendicular to the axis, for which purpose the plates had been pierced in the centre. Consequently the plates were exposed at an angle of 45° with the horizontal surface. This angle ensures the maximal
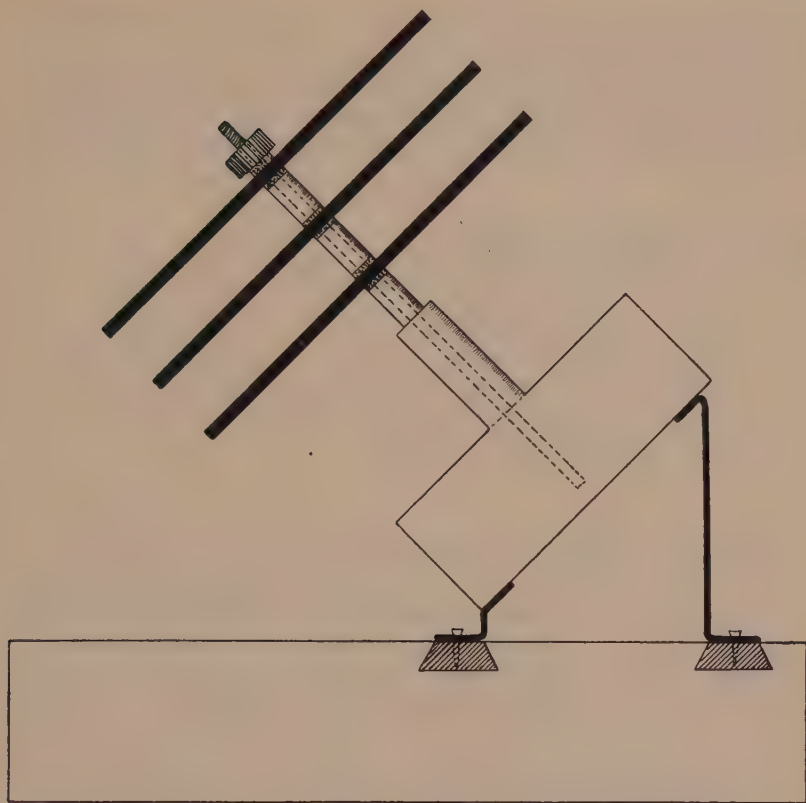
Figure 24. Time-piece apparatus. (Schematic).

orientation of the spat and a far better spatfall than may be expected if the angle is greater (e.g. 90°).

As the axis completes one revolution in 12 h. 25 m., the plates turn round once during each tidal cycle. If we place the plates on the apparatus with one particular side uppermost, we shall find the same side uppermost when we return the next day or after some days at the same stage of the tidal cycle, leaving out of account the rather slight differences between the duration of the actual tide and the average duration of the tidal cycle (i.e. 12 h. 25 m.). The entire apparatus was covered with wire netting to keep off floating sea-weed.

Though several series caught but little spat and some series miscarried on account of the water that penetrated into the box

through the packing of the axis, I yet obtained some successful
sets of plates with this apparatus.

I caught 174 spat on three plates placed on this apparatus
at the station Kattendijke from 8–14 July 1939. The orientation
of this spat has been visualized in the following diagram:

Ebb

26 34   L.W.

12   32

22   7

20 21

H.W.   Flood

Kattendijke
8—14 July 1939
Time-piece apparatus
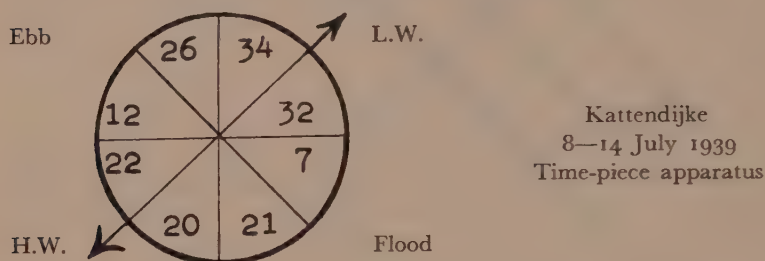
The arrows indicate the direction in which the majority of
the umbos should point if all the spat has attached during low
slack water (L.W.) or during high slack water (H.W.). Although
this was only a preliminary experiment, the results seem to
indicate that at the station Kattendijke setting is most frequent
at low slack water, while a maximum of less importance seems
to occur at high slack water.

As not all the spat attaches with the umbo pointing precisely
in the direction of preference, my data are as yet insufficient
to enable me to determine the precentage of spat attaching at
low slack water; so it is only possible for me to indicate a maxi-
mum of setting during the last-named part of the tidal cycle.

These results are in accordance with the other data concerning
this station. At Kattendijke the number of larvae is several
times greater in the hours roundabout low water than round-
about high water, as has been discussed in a preceding section
(fig. 12). The number of full-grown larvae is likewise greatest
at low water, but their number does not vary so much in the
course of the tidal cycle as that of the smaller larvae, seeing
that the larvae present here during high water are practically
all of them in later developmental stages (fig. 12).

The currents are fairly strong at this station; during the height
of the tide velocities from 100 to 150 cm/sec. may be recorded.
This hinders attachment during an important part of the tidal

cycle. Attachment during these stages of the tide is only possible in sheltered places. Consequently maxima of setting are to be expected here during slack water; the largest maximum during low slack water, as the number of full-grown larvae is greatest then. The results obtained with the time-piece apparatus are exactly those that were expected.

I collected other series with this apparatus the at station Yersche Bank. The results obtained indicate that the intensity of setting is not uniform during the entire tidal cycle at this station either.

Period	Direction of Umbos (8 sectors)							
	NNE	ENE	ESE	SSE	SSW	WSW	WNW	NNW
26 VII– 1 VIII 1939	11	25	20	18	34	64	29	24
16 VIII–19 VIII 1939	8	17	12	19	19	26	29	21
19 VIII–25 VIII 1939	24	24	34	51	109	81	45	40
25 VIII–28 VIII 1939	9	11	7	5	15	14	8	8
Total	52	77	73	93	177	185	111	93

The total numbers recorded in another form yield the following diagram:

Ebb   L.W.   93   52   111   77   185   73   177   93   H.W.   Flood
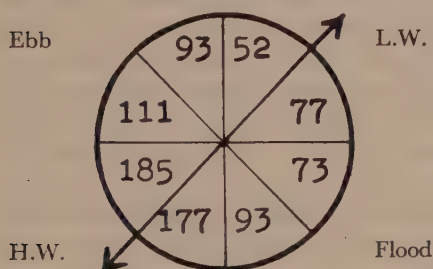
Yersche Bank
Time-piece apparatus

These preliminary data indicate that at the station Yersche Bank the intensity of setting is greatest at high water. This is in accordance with the results of the exchanging-experiment carried out at this station.

What factors may be the cause of there not being a marked setting maximum at low slack water?

The number of full-grown larvae per unit of water does not vary much here in the course of the tidal cycle (fig. 11), but the total volume of water and consequently the total number

of full-grown larvae is much greater at high water than at low water at this station, which may account for a difference in setting at high slack water and low slack water. Moreover in shallow places there is a tendency on the part of the current-velocity near the bottom to be insignificant for a shorter period at low water than at high water, which is connected with the height of the moving water-masses. I dare not yet decide whether these two factors are in themselves sufficient to account for the absence of a marked setting maximum at low slack water.

It is my intention to carry out further experiments with the time-piece apparatus in the next season.

XXX. VERTICAL DISTRIBUTION OF THE SPATFALL

In recent years several investigators have investigated the vertical distribution of the spatfall. The results of their investigations clearly show how injustifiable it is to base general conclusions and practical advice on accidental field observations.

Statements like that of KÄNDLER (1930): ,,Die Austernlarve heftet sich meist an der Unterseite der Gegenstände fest und tut dies mit Vorliebe in Nähe der Wasseroberfläche falls sie hier Ansatzkörper vorfindet", and that of ORTON (1937 a): ,,Larvae of all kinds of oysters will therefore settle when they are ready at any depth in the water or on the bottom and although tradition and economic necessity may have imposed bottom collection, there is no reason why good spat-collections may not be made in these regions on suitable material suspended in the water at suitable depths", are not based on the results of deliberate experiments.

HOPKINS (1937), experimenting on *Ostrea lurida*, filled wire baskets with clean shells, suspended them in series one above the other and hung the entire series from a float. In all cases the samples from the surface (0–15 cm) caught least spat, possibly, in HOPKINS' opinion, because of the scouring action of the waves.

The samples from depths of 0,15 to 2,00 metres contained about equal numbers of spat. Although the bags suspended near the surface appeared to have caught somewhat more spat than those at greater depths, I do not believe that the differences are great enough to admit of reliable measurement with the methods used by HOPKINS (shell-collectors).

BONNOT (1936) stated that in Humbolt Bay there appeared to

be no appreciable difference between the spatfall of *Ostrea lurida* near the surface and somewhat deeper.

Floats filled with shells or manufactured collectors (e.g. egg-case fillers) are in use on a commercial scale in the Puget Sound, not only because of the somewhat greater spatfall near the surface, but also owing to a shortage of suitable breeding-grounds for the planting of collectors. The deep channels here appeared to be unsuitable for spat-collection.

Other investigators have recorded or recommended the use of floats filled with collector-material for other kinds of oysters. Thus NEEDLER (1933) describes the use of floats in the spat-collection of *Ostrea virginica* in Malpeque Bay, which were introduced there after he had convinced himself (1932 a) that the optimum setting occurs here just below the low-water mark. NEEDLER tells us that the advantages attending the use of floats are the absence of starfish and of smothering silt and sand.

BONNOT and MAC MILLAN (1931) advised oyster-farmers to make attempts to collect the spat of *Ostrea gigas* with the aid of tarred rope suspended from floats after the example of the Japanese culture-methods practised on this kind of oyster.

SEKI and TANAKA (1931) hung tile collectors from the surface to the bottom and found that the most favourable layer for attachment of *Ostrea denselamellosa* was situated 0,6 metre above the bottom.

VILLALUZ (1939) ascertained the vertical distribution of the spatfall in Bacoor Bay (Philippines). He does not tell us which of the 18 species of oysters occurring in the Philippines was studied. He used string collectors consisting of oystershells strung on a piece of galvanized iron wire. As the shells differed somewhat in size and shape and were moreover not held at precisely the same angle of surface and as the periods of exposure were generally quite long, his methods can hardly be called quantitative. The bottommost shells caught no spat, probably because of a contact with the soft mud-bottom. The best spatfall was obtained about halfway between bottom and surface. VILLALUZ does not give any information about the local hydrographical conditions.

PRYTHERCH (1929, 1934) compared the vertical distribution of the spatfall of *Ostrea virginica* in several localities. In Great South Bay the optimum spatfall occurs near the bottom, in Beaufort (Sth. Carol.) from 3 to 5 feet above the low-water mark and in Long Island Sound from 1 foot above to 1 foot

below the low-water mark. PRYTHERCH points to the great difference in hydrographical conditions in these different localities. He concludes that the heaviest spatfalls may be expected to occur at the levels where at the moment the current reaches its minimum velocity the larvae are most abundant. No other investigators mention the great influence current-velocities may have in determining the vertical distribution of the spatfall in co-operation with factors, such as the distribution of the larvae. Attachment of *Ostrea virginica* is even possible at a depth of 70 feet, but it is usually heavier in shallower water (HIGGINS 1937).

Apart from the remarks of KÄNDLER and ORTON quoted above, it is only COLE and KNIGHT JONES (1939) who inform us about the vertical distribution of the spatfall of *Ostrea edulis* (under semi-natural conditions). Their towers of curved limed tile-collectors, built up from the tank bottom (depth 6 feet) to the surface, showed no constant variation in the intensity of setting from bottom to surface, which led them to the belief that larvae settle equally readily at all depths in the tank.

In 1938 they hung out shells at three different levels and caught far more spat on the shells at the surface than on those at the other levels. They were, however, able to reconcile those two facts by considering that oysterlarvae swim vertically upwards only and that lateral movements of any importance, which are only produced by the motion of the medium, are practically absent in tanks. In consequence of this it must be difficult for the larvae to reach the piled-up tiles. Although COLE and KNIGHT JONES admit that the number of larvae reaching the collectors may be commensurate with the height of the column of water below the collectors (owing to the vertical swimming habit) and that moreover the larvae are somewhat more abundant in the upper half of the tank than in the lower half, these investigators yet assume that these phenomena are not in themselves sufficient to account for the high intensity of setting observed on the under-surfaces of shells held near the surface. They conclude that the larvae of *Ostrea edulis* probably prefer to attach near the surface and that „on the natural beds, where there is every reason for supposing that larvae will behave in the same way[1]), recognition

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[1]) Spacings are mine.

of the fact that spat set most readily near the surface opens
the possibility of utilizing floating trays of cultch for the collec-
tion of spat." They even say that, as larvae are abundant on
the British oysterbeds (according to ORTON), heavy spatfalls
· might be obtained there on shells held in floating trays.

I will presently show how injustifiable it is to draw general
conclusions from results obtained under semi-natural conditions.

I used the following device to investigate this matter in the
Oosterschelde. A long pole (8 metres), such as are in use as sea-
marks here, was fixed into the sea-bottom. Brass frames, in
which the coated glass plates had been made to fit, were attached
in a horizontal position to a stout lath, carrying a heavy (20 kg)
foot of reinforced concrete. The plates were placed in the frames,
the lath was planted on the sea-bottom right next to the sea-
mark and the topmost part of the lath was attached to the
sea-mark with a piece of rope. The plates were exchanged
regularly.

One of these devices was planted at the Yersche Bank in the
station 392 (fig. 1), where the depth is about 3 metres at low
water, so that one or two of the plates became exposed during
low water. The plates were fully exposed to the currents.

Periods of exposure	Distance of the plates from the bottom							
	25cm	75cm	125cm	175cm	225cm	275cm	325cm	375cm
15 VII–24 VII 1938	17	2	7	6	2	0	0	0
24 VII–30 VII 1938	30	6	4	4	0	0	0	0
30 VII– 5 VIII 1938	11	2	1	4	0	1	0	0
5 VIII–11 VIII 1938	326	76	98	54	89	57	8	1
11 VIII–23 VIII 1938	64	14	35	23	18	7	4	1
5 VII–11 VII 1939	21	6	4	1	—	0	0	0
11 VII–17 VII 1939	38	22	—	0	—	0	0	0
17 VII–23 VII 1939	26	12	—	1	0	0	—	—
23 VII–29 VII 1939	20	2	—	2	0	0	—	—
29 VII– 7 VIII 1939	39	13	23	—	0	—	—	—
7 VIII–13 VIII 1939	26	11	3	—	1	—	—	—
13 VIII–19 VIII 1939	22	13	4	—	0	0	—	—
19 VIII–25 VIII 1939	23	15	14	—	0	0	—	—
Totals	663	194	193	95	110	65	12	2

A similar apparatus was placed at the station Kattendijke,

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in a place where the depth is 4 metres at low water, so that the topmost plate seldom or never became exposed at low tide.

Periods of exposure	Distance of the plates from the bottom							
	25cm	75cm	125cm	175cm	225cm	275cm	325cm	375cm
5 VII–11 VII 1939	14	15	9	1	0	1	0	0
11 VII–17 VII 1939	27	23	7	9	15	5	2	0
17 VII–23 VII 1939	27	20	9	5	6	7	5	1
23 VII–29 VII 1939	10	11	8	2	4	1	2	1
29 VII– 7 VIII 1939	30	5	10	8	9	5	–	–
7 VIII–13 VIII 1939	14	10	5	5	12	6	4	0
13 VIII–19 VIII 1939	2	2	4	2	0	3	1	0
Totals	124	86	52	32	46	28	14	2

A similar device was placed at the station Bergsche Bank (fig. 1), where only the topmost plate became exposed at low water.

Periods of exposure	Distance of the plates from the bottom						
	25cm	75cm	125cm	175cm	225cm	275cm	325cm
5 VII–14 VII 1939	6	13	2	5	3	0	0
14 VII–17 VII 1939	6	1	3	0	0	0	0
17 VII–23 VII 1939	4	1	1	0	0	0	0
23 VII–29 VII 1939	7	2	0	3	0	0	0
29 VII– 7 VIII 1939	12	3	2	4	1	0	0
7 VIII–16 VIII 1939	6	3	2	0	1	–	1
16 VIII–22 VIII 1939	11	8	10	1	7	–	0
Totals	52	31	20	13	12		1

I also used another kind of device at the station Yersche Bank 392, consisting of a floating buoy [1]) from which a stout lath, carrying 6 frames attached to it in a horizontal position, had been suspended. The end of the lath carried a heavy foot of reinforced concrete, weighing about 20 kg, with a view to keeping the lath in a vertical position. In this way the plates were held at a constant distance from the surface, while the other device ensured a constant distance from the bottom. With

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[1]) The buoy was anchored to prevent it from being carried away by the currents.

this floating apparatus I obtained 11 series of plates in the course of the summers of 1938 and 1939. Only one of these series appeared to have caught a moderate number of spat, viz. the series that had been exposed during the very intense spatfall in August 1938.

Periods of exposure	Distance of the plates from the surface					
	25 cm	75 cm	125 cm	175 cm	225 cm	275 cm
5 VIII–11 VIII 1938	15	17	35	32	45	63

My field observations in the Oosterschelde clearly demonstrate that the heaviest setting occurred near the bottom. Floating collectors that had been fully exposed to the currents yielded only a mere sprinkling of spat. This phenomenon cannot be ascribed to differences in the vertical distribution of the full-grown larvae, as my data convincingly show that the vertical distribution of the larvae is quite uniform during the entire tidal cycle (fig. 11). Hence it follows that the larvae do not sink to the bottom in consequence of their growing weight, as has often been supposed.

I ascribe the differences in the vertical distribution to the influence of the currents on the setting process. I have shown in a preceding section that setting is more intense when the plates are sheltered from the currents. The current-velocity is far slower near the bottom than in the other water-layers. In the Oosterschelde the greatest current-velocities are recorded near the surface. Owing to the friction against the bottom the current slackens in the bottom layers. Consequently the period favourable for attachment is far shorter in the upper layers of the water than near the bottom, as in situations remote from the bottom crawling on exposed surfaces is possible during a very short part of the tidal cycle only, owing to differences in current-velocity.

My data support the view of PRYTHERCH that a heavy spatfall is likely to occur at those levels where larvae abound at the moment that the currents are negligible.

It will be clear from the foregoing that the occurrence of intense spatfalls near the surface in tanks does not imply that the same phenomenon will be observed under field-conditions, except perhaps in bodies of water where the currents are negligible throughout the tidal cycle.

As the larvae in the Oosterschelde are distributed in the water uniformly, I think it would be possible to catch more spat than I did in places remote from the bottom by sheltering the cultch from the currents, for instance by using wire bags with shells, but this does not alter the fact that current conditions are more favourable near the bottom.

In 1938 we constructed a solid raft, attached limed tiles on it and bags of shells and anchored it above the oysterbeds. The results were disappointing. All the cultch-material appeared to be covered with a heavy layer of barnacles and the oyster-spat was very scarce. I ascribe these poor results not merely to the influence of the current-velocity, but I am inclined to believe that especially the exceptionally heavy set of barnacles must have hindered the spatfall and have overcrowded the spat.

The fact that in the Puget Sound HOPKINS did not find a distribution of the spatfall similar to that in the Oosterschelde does not necessarily point to a different behaviour of the larvae of *Ostrea lurida*, but may perhaps be ascribed to different current conditions there. Moreover it should be remembered that HOPKINS does not tell us how the larvae are distributed in the water of the Puget Sound. The practical absence of spatfall in the deep channels in the Puget Sound is perhaps also to be explained by the influence of the currents on the spatfall.

XXXI. THE HORIZONTAL DISTRIBUTION OF THE SPATFALL

It is a striking fact that practically every field investigator states that there are places where the spatfall may be profuse, while other places, though often quite near the former, yield only a mere sprinkling of spat.

The suitability for setting is evidently determined by an optimum combination of environmental factors. The number of mature larvae in the water is one of the most important of these factors. Several investigators neglect the influence of other factors on the horizontal distribution of the spatfall and try to correlate the observed differences with differences in the horizontal distribution of the larvae. Thus NELSON (1921) states that the intensity of the spatfall is commensurate with the number of larvae present in the places concerned. He advises that the number of larvae per unit of water should be ascertained in order to determine the suitability for attachment

of the places in question. NELSON observed profuse setting in places where the currents are slow or where eddies occur. The same observation has been made by CHURCHILL and GUTSELL (1921). Neither they nor NELSON have interpreted this phenomenon as an effect of the influence of the currents on the setting process, but merely as an outcome of differences in the horizontal distribution of the larvae. The fact that there are places, which, though fairly remote from the centre of larvae-production, yet show an abundant spatfall, has been ascribed by them to a local secondary accumulation of the previously dispersed larvae. They do not make clear, however, the nature of the mechanism that may be supposed to bring about such an accumulation and they have not yet supplied any data to prove the actual occurrence of the accumulation assumed by them.

It will be clear from the foregoing that the slow currents in places where eddies occur bring about favourable setting conditions, so that setting may be profuse there, while in places near by, where the number of larvae may be the same, we shall find but a mere sprinkling of spat, as strong currents hinder the setting for the greater part of the tidal cycle there.

The number of larvae per unit of water is a factor that should not be neglected, however, and I do not agree with authors like BONNOT (1936), who assume that the number of mature larvae in the water cannot give us any indication of the number of spat that is going to settle.

In the preceding section I have already discussed PRYTHERCH'S statement (1929, 1934) that the heaviest setting is likely to occur in places where the larvae are plentiful at the moment that the current reaches its minimum velocity. PRYTHERCH also states that the best spatfall occurs in the centre of the natural oysterbeds, but perhaps it would be more exact to say that the centre of natural oysterbeds is situated in those places where the combination of all the factors concerned ensures the most favourable conditions for setting and survival.

Eddies and other bodies of relatively still water also appeared to be favourable for the setting of *Ostrea edulis*. KÄNDLER (1928, 1930) and ORTON (1937 b) did not ascribe this to the influence of the current-velocity on the setting process, but they suppose that the larvae are accumulated in such places. BOURY (1929 b)

tried to account for the fact that some parts of the beds are more favourable for spatfall than others, but he did not compare the number of larvae, nor did he ascertain the current conditions, so that we are not surprised at his inability to explain the difference.

Just as in other centres of spatproduction marked differences in the spatfall were found to occur at different stations in the Oosterschelde. I soon found out, however, that the occurrence of young oysters is no reliable standard for the intensity of setting, as marked differences in the local death-rate were found to occur. In some places with a heavy spatfall we find but little spat in autumn, as the conditions for survival are unfavourable there owing to smothering by silt or sand or to an abundance of enemies, such as young starfish.

In using the method of ascertaining the spatfall with the aid of coated glass plates placed in containers of reinforced concrete the influence of the death-rate is eliminated. With this device we caught the following number of spat (the plates were exposed for periods of three days):

Periods of exposure	Yersche Bank	Strijen	Berg-sche Bank	Goris-hoek	Katten-dijke	Wemel-dinge
8 VII– 3 IX 1935		357			361	
24 VI–17 VIII 1936	180	56	49	28	39	111
16 VI– 2 IX 1937	447	175	86		272	342
24 VI–29 VIII 1938	1053	383	171		357	697
23 VI–31 VIII 1939	629	351	109		385	433

There is not much difference in the number of full-grown larvae in the course of the tidal cycle at the stations Yersche Bank, Strijen, Gorishoek and Bergsche Bank.

Current conditions are, however, by far the most favourable at the station Yersche Bank, a shallow place in the centre of the basin of the Oosterschelde, where the currents are far slower than in the deeper channels near by. Strong currents prevail at the station Bergsche Bank, so that the period during which attachment is possible is very short there. Hence the poor spatfall. The station Strijen is situated on the slope of a deep channel in which swift currents prevail, but as the container is sheltered to some extent behind a small breakwater, the number of larvae caught is rather satisfactory here, though

far smaller than at the station Yersche Bank. The number of mature larvae at the station Gorishoek does not differ much from that at the other stations in the basin, but as the currents are very strong here this station is nonetheless unsuitable for spat collection. Just in the lee of a dike at Gorishoek the water is relatively still and tile-collectors placed there in 1939 caught a great number of spat.

At the stations Wemeldinge and Kattendijke conditions are different. The number of larvae at these stations is far greater during low water than during high water, as has been described above. As the station Wemeldinge is not so remote from the basin as the station Kattendijke, the period during which the basinwater (containing a great number of larvae) is present is of longer duration at the station Wemeldinge than at the station Kattendijke. This may explain the difference in spatfall, current conditions being the same at these stations.

From the foregoing I conclude that the two most important factors in determining the suitability for spatfall are the number of mature larvae in the course of the tidal cycle and the current-velocities during that period. This view, which is in accordance with that of PRYTHERCH, is supported by what we know of the distribution of the spatfall in the Oosterschelde, which spatfall was observed both on practical collectors and on the plates placed in containers for periods of three days. In giving advice as to places suitable for spat-collection on a commercial scale, it is not sufficient to know the current conditions and the relative abundance of the larvae, but we also ought to know if the bottom is suitable for the planting of collectors and if smothering by silt or sand or ravages by animal enemies are not too considerable.

XXXII. PROPERTIES OF THE COLLECTOR-MATERIAL AND THEIR INFLUENCE ON THE FREQUENCY OF SETTING

The influence of the angle of surface

Oyster-growers have often noticed that most of the spat of *Ostrea virginica* and *Ostrea lurida* is to be found on the under-surfaces of collectors. NELSON (1926, 1927) compared the frequency of setting on uppersides and undersides of shells and found more than 10 times as much spat on undersides than on uppersides. His shells were exposed for short periods, so that

this phenomenon cannot be explained by a decreased suita-
bility for setting on the upper-surfaces caused by a deposition
of silt or by algal growth. NELSON ascribed this difference to
the influence of light on the setting process. He assumed that
light stimulated the larvae to swim and that they become
quiescent and proceed to set in shady situations, such as the
undersides of the collectors. NELSON stated in a previous paper
(1921) that his observations had shown him that the pull
of gravity has practically no influence in determining the
position of attachment of oysterlarvae.

PRYTHERCH (1934 a) studied the spatfall in vitro and observed
that the vertical surfaces of his containers carried far more
spat than their horizontal bottom-surfaces.

The first deliberate field-experiments to investigate this
matter were carried out by HOPKINS (1935, 1937), who experi-
mented with *Ostrea lurida*. He eliminated the influence of
sedimentation and algal growth by exposing his glass test-plates
for short periods (24 h.) He exposed his plates at different angles,
which have been referred to as follows:

    0° under-horizontal   45° under-surface of a 45° plate
  180° upper-horizontal   135° upper-surface of a 45° plate
   90° vertical

HOPKINS obtained the following results with *Ostrea lurida*
(number of spat per 2400 square inch):

0°	45°	90°	135°	180°
1195	181	11	3	1

These results led to the construction of a special type of
collectors of cement-coated cardboard. This collector provided a
great many horizontal surfaces and was moreover less subject
to silting of its partitions than egg-case fillers, another collector
of cement-coated cardboard. HOPKINS' special design appeared
to be more than three times as effective per unit of surface
than the standard egg-case fillers introduced in oyster-culture
by PRYTHERCH. In consequence of the rather rough surfaces
of the cement-coated cardboard the vertical walls have a rather
large horizontal component, so that the numbers of spat on
the surfaces exposed at different angles are less divergent than
in the case of the smooth glass plates.

HOPKINS does not ascribe the enormous difference in spatfall

on the surfaces held at different angles to some geotropic reaction of the larvae or to a discriminating selection of the angle of surface by the larvae, but he puts foreward a purely mechanical explanation based on the accidental contact of the foot of the larvae with the substratum as a result of their vertical swimming habit. The larvae swim with velum and foot turned upwards and therefore HOPKINS assumes that "it is most likely that the swimming larva, as it comes into contact with a surface from below, is able to hold on it with the foot, while on coming down upon a surface it is the hinge portion of the shell that touches. In this manner, as the angle of surface departs more and more from the under-horizontal, there is constantly less chance of its foot touching."

If this interpretation is correct, it is difficult to explain why surfaces held at an angle of 45° catches far less spat than under-horizontal surfaces, instead of the proportion being about 3 : 4 (sin. 45°)!

Other observations on *Ostrea lurida* have been made by COE and ALLAN (1937), who found more spat on the undersides of their collectors than on the vertical sides and by BONNOT (1937 a, 1937 b). BONNOT ascertained the spatfall on a special type of manufactured collector consisting of strips of cement-coated plywood placed above each other in a horizontal position, with interspaces of 3/4 inch. The largest spatfall occurred on the upper-surfaces. BONNOT explains this phenomenon by ascribing it to the considerable friction in the interspaces of his collectors, which causes the water to roll and swirl. The larvae are unable to maintain their normal swimming position, many of them are even presumed to be turned over, so that "larvae are carried along in all positions and it would seem logical that the slight pull of gravity would cause more of them to rest on the surface which was below them."

SCHAEFER (1937) investigated the influence of the angle of surface on the intensity of setting in *Ostrea gigas*, using the same device as HOPKINS. He counted per 2400 square inches of smooth glass:

0°	45°	90°	135°	180°
346	119	35	21	6

These results are essentially the same as those obtained by

Hopkins for *Ostrea lurida*, although for *Ostrea gigas* the proportions are less excessive. Schaefer is inclined to believe that Hopkins' explanation does not cover all the facts and that a negative geotropism is likely to play a part in the setting of these kinds of oysters. Schaefer's results do not tally, however, with those obtained by Yokota (1936) and Miyazaki (1938). Yokota compared the spatfall of *Ostrea gigas* on shells held at different positions and found that upper-surfaces catch more spat than under-surfaces. Shells are, however, less suitable to this purpose, as they do not provide plane surfaces. Yokota also observed that more spat settled on the bottom of a glass container (180°) than on its vertical walls (90°). Miyazaki exposed calcareous plates in a horizontal position at different depths in order to study the season of attachment of various sedentary organisms. He found that 4 to 8 times as many spat of *Ostrea gigas* settled on the upper-surfaces of his plates than on the under-surfaces.

Spat of *Ostrea edulis* has often been observed in the field attached to under-horizontal surfaces (Kändler, 1928, Havinga, 1929, Hagmeier, 1930, Gaarder and Bjerkan, 1934). As the objects to which this spat was attached had been in the water for a considerable time, we are not justified in concluding from these observations that *Ostrea edulis* sets more frequently on under-surfaces than on upper-surfaces, seeing that factors like algal growth, sedimentation and differences in the death-rate have not been eliminated.

Cole and Knight Jones (1939) used Hopkins' device in their tanks. They used slates instead of glass plates, as it is difficult to induce larvae of *Ostrea edulis* to set on smooth glass. Their results were:

0°	45°	90°	135°	180°
12407	6123	119	316	232

These results seem to tally with those of Hopkins and Schaefer concerning *Ostrea lurida* and *Ostrea gigas*. Vertical surfaces appeared, however, to catch less spat of *Ostrea edulis* than surfaces held at angles of 135° and 180°, which was not the case with the other kinds of oysters. Cole and Knight Jones do not believe that Hopkins' explanation, based on the

vertical swimming position of the larvae, covers all the facts, for the plates held at an angle of 45° caught only about half the number of spat that was counted on under-horizontal surfaces (0°), instead of about 3/4 (sin. 45°). They are inclined to suppose that the crawling larvae must excercise a more or less discriminating selection of the angle of surface.

I have gathered many data on the influence of the angle of surface in the Oosterschelde. All the plates I used for ascertaining the intensity of setting in the course of the season of reproduction were exposed at angles of 45°, so that from the data thus obtained I can also study the difference in spatfall between surfaces at 135° and 45°. Moreover I constructed a special container in which 3 plates could be held in a vertical position and 3 in a horizontal position (fig. 18). This device was used in the seasons of 1938 and 1939. In 1939 I used moreover a container in which 12 plates could be exposed at different angles, so that the entire set was arranged in the shape of a fan. I adopted the same notation as has been used by the other authors, i.e. the notation by which the upper-surfaces of a plate inclined at an angle of 45° are considered as being at an angle of 135° with the horizontal and other surfaces accordingly.

The plates used for ascertaining the intensity of setting yielded the following data:

Sea-sons	Yersche Bank		Kattendijke		Wemeldinge		Strijen		Bergsche Bank	
	45°	135°	45°	135°	45°	135°	45°	135°	45°	135°
1935			147	541			233	413		
1936	120	343	30	82	125	220	65	101	43	104
1937	260	1181	173	551	342	650	188	329	88	166
1938	554	2607	324	747	748	1338	475	682	264	249
1939	231	1656	288	866	393	886	306	757	107	220
Totals	1165	5787	962	2787	1608	3094	1267	2282	502	739

In 1938 the coated plates from the container with horizontal and vertical plates yielded the following figures:

Periods of exposure	Horizontal plates						Vertical plates					
	topm. pl.		2nd. pl.		3rd pl.							
	0°	180°	0°	180°	0°	180°	90°	90°	90°	90°	90°	90°
3 VII-12 VII	10	69	15	30	25	40	5	2	6	7	5	7
12 VII-21 VII	12	53	7	23	7	36	3	2	4	8	8	18
2 VIII- 8 VIII	45	224	–	–	37	70	18	9	7	16	27	14
8 VIII-14 VIII	136	1194	–	–	71	1031	89	59	96	99	124	78
Totals	203	1540			140	1171	115	72	113	130	164	117

Averages:

0°	90°	180°
171	118	1358

In 1939 the same device yielded:

Periods of exposure	Horizontal plates						Vertical plates					
	topm. pl.		2nd. pl.		3rd pl.							
	0°	180°	0°	180°	0°	180°	90°	90°	90°	90°	90°	90°
2 VII- 8 VII	23	75	15	35	19	30	3	10	9	4	13	9
8 VII-14 VII	33	127	31	40	36	28	4	13	10	14	13	12
14 VII-20 VII	33	136	43	139	38	109	9	15	16	12	15	16
20 VII-26 VII	18	113	16	36	39	48	4	8	14	17	9	21
26 VII- 4 VIII	27	75	29	76	39	103	12	9	15	8	27	11
4 VIII-10 VIII	38	82	35	23	19	17	20	11	5	11	7	5
10 VIII-16 VIII	23	48	21	29	43	64	17	8	8	10	10	6
16 VIII-22 VIII	34	104	27	98	42	105	34	20	41	17	44	26
Totals	229	760	217	476	275	504	103	94	118	93	138	106

Averages:

0°	90°	180°
240	109	580

The fan-shaped set of plates caught in 1939:

Periods of exposure	0°		22½°		45°		67½°		90°				112½°		135°		157½°		180°	
VII – 8 VII	20	10	22	13	13	7	2	5	6	1	10	4	23	34	54	61	34	38	24	35
VII – 14 VII	12	10	16	16	18	23	9	5	7	4	7	0	37	21	20	45	59	41	27	58
VII – 20 VII	35	13	32	27	17	11	10	4	16	12	13	6	56	25	106	48	130	70	29	57
VII – 26 VII	27	27	26	22	22	22	10	7	13	7	10	8	39	26	46	60	55	32	27	25
VII – 4 VIII	5	14	28	37	15	21	9	14	8	11	1	2	50	31	76	104	77	87	26	15
VIII – 10 VIII	15	3	23	17	5	6	8	6	4	3	7	5	28	18	48	15	41	30	7	10
VIII – 16 VIII	11	6	17	22	9	14	6	4	5	5	11	6	29	25	19	35	25	38	9	12
VIII – 22 VIII	26	34	20	34	17	11	4	6	11	4	12	4	26	42	72	21	52	72	31	12
Totals	151	117	184	188	116	115	58	51	70	47	71	35	288	222	441	389	473	408	180	224

Averages:

0°	22½°	45°	67½°	90°	112½°	135°	157½°	180°
134	186	115	55	56	250	415	441	202

During the same periods that the container with horizontal and vertical plates was used, the ordinary container at the same station caught per plate:

	135°	45°
1938	633	140
1939	478	67

The following are the data obtained at the station Yersche Bank, combined in one table:

	0°	22½°	45°	67½°	90°	112½°	135°	157½°	180°
Ordinary plates (1936–1939) .			1165				5787		
H.V. plates + Ord. plates 1938	171		140		118		633		1358
H.V. plates + Ord. plates 1939	240		67		109		478		580
Fan-container 1939	134	186	115	55	56	250	415	441	202
Special horizontal series 1939.	771								2370

I conclude from these data that under field-conditions in the Oosterschelde upper-surfaces collect more spat than under-surfaces and that vertical surfaces are least suitable for attachment.

These data differ greatly from those obtained by HOPKINS, who

experimented likewise under field-conditions, albeit with *Ostrea lurida*. As the larvae of *Ostrea edulis* show the same swimming position as those of other kinds of oyster, viz. with the velum upwards, HOPKINS' interpretation, based on the chance of the foot touching an object, cannot be correct. The great difference between the number of spat on surfaces held at 0° and at 45° in his experiments points in the same direction. It is not easy to give a plausible explanation of this difference in setting of these closely related species.

COLE and KNIGHT JONES obtained results which are quite different from mine, but then they carried out their experiments in tanks (so under semi-natural conditions), where horizontal currents are practically absent, so that the larvae can practically move in a vertical direction only, while under field-conditions horizontal movements are far more considerable than vertical. In consequence of the absence of horizontal currents in tanks it is very difficult for the larvae to reach the uppersides of objects, especially when these objects are exposed near the surface. If the conclusion from my experiments, that the larvae of *Ostrea edulis* prefer to attach on upper surfaces, is correct, it remains possible that in tanks under-surfaces collect far more spat than upper-surfaces, owing to the limited possibilities of movement in them. It is my belief that the fact that vertical surfaces caught less spat than upper-surfaces in COLE's experiments indicates that his results are in better accordance with mine than with those of HOPKINS.

The preference of *Ostrea edulis* for certain angles of surface is by no means so pronounced as in *Ostrea lurida*, although I was able to prove in the section on the orientation of the spat that the full-grown larvae of *Ostrea edulis* are extremely sensitive to the pull of gravitation.

The difference in spatfall on upper- and under-horizontal surfaces varied somewhat. On an average upper-surfaces of horizontal plates caught from 2 to 3 times as much spat as under-surfaces (under field-conditions). Upper-horizontal surfaces often caught more spat than surfaces exposed at an angle of 135°.

It is my belief that in the container in which the plates were held in a fan-shaped arrangement other factors must have interfered. The plates in this container stood close together and although the side-walls were amply provided with oblong holes

(c.f. fig. 19), it remains possible that current conditions were not quite the same for the various plates. Consequently I do not think that we are justified in deducing from the data obtained with this device that surfaces exposed at an angle of $157\frac{1}{2}°$ are more suitable than those held at an angle of 180°.

Although current-velocities are somewhat greater in the Oosterschelde than in the Puget Sound, it is impossible to ascribe the differences between HOPKINS' data and mine to this. For also in those cases where the plates were exposed in such a way that current conditions were exactly the same for upper-surfaces and under-surfaces (I am referring to the plates used for ascertaining the vertical distribution of the spatfall) I found more spat on upper than on under-surfaces (1190 : 479).

From my first table in this section it may be seen that the proportion between the intensity of setting on surfaces held at an angle of 135° and of 45° was about 5 : 1 at the station Yersche Bank, 3 : 1 at Kattendijke, 2 : 1 at the station Wemeldinge and less than 2 : 1 at the stations Strijen and Bergsche Bank.

The strongest currents occur at the stations Bergsche Bank and Strijen, while the currents are relatively slow at the station Yersche Bank. It is possible that the correlation between the current-velocity and the proportion of spat on upper- and under-surfaces of plates held at an angle of 45° is an indication that under-surfaces are somewhat better protected from the currents than upper-surfaces, though we tried to eliminate this factor by piercing the side-walls of the containers (fig. 16).

It may also be seen from one of my tables in this section that the upper-surface of the topmost of a set of three horizontal plates caught but slightly more spat than the upper-surfaces of the other two plates, although the upper-surface of the topmost plate was more easily accessible for the larvae than were the other two. It should be borne in mind, however, that the active vertical movements of the larvae are far slower than the passive horizontal, so that under field-conditions the chance of their reaching the second and the third plate is not so much smaller than the chance of their reaching the topmost plate.

Summarizing, I can state that my experiments demonstrate that the larvae of *Ostrea edulis* do not show the same behaviour with regard to the angle of surface at which the collectors are

exposed as those of *Ostrea lurida*. Upper-surfaces catch more spat than under-surfaces and vertical surfaces collect least spat. Hopkins' interpretation of the behaviour of *Ostrea lurida* cannot be correct. The difference between my data and those obtained by Cole and Knight Jones may be ascribed to the limited possibilities for horizontal movements in the still water of the tanks.

I am inclined to ascribe the difference in spatfall on surfaces held at various angles to a discriminating selection on the part of the larvae and I reject a purely mechanical explanation. This selection may be interpreted as a result of the efforts of the mature larvae to attain their ecological norm.

Cole says that the larvae of *Ostrea edulis* set most frequently near the upper free edges of the undersides of slates held at an angle of 45°, which, according to him, points to a tendency to crawl upwards against gravity on inclined surfaces. I have never yet observed this phenomenon in the Oosterschelde.

The influence of the colour of the substratum

Folpmers (1924) presumed that oysterlarvae prefer a dark substratum for attachment: „Het zwemmende oesterbroed zoekt toch bij voorkeur het donker op en is afkeerig van veel licht." He compared the spatfall on black and white tile-collectors, but he counted only 3 to 4 spat per tile, which numbers are far too small to base conclusions on.

Another experiment with coloured tile-collectors has been carried out by Thieblemont (Herman 1937). He counted the number of spat on his tiles at the end of the season of repro- duction and found on an average 7,7 spat on white tiles, 6,8 on blue tiles, 3,7 on green tiles, 3,6 on red tiles, 3,1 on violet tiles, 2,2 on black tiles and 1,7 on yellow tiles. He did not eliminate differences in the death-rate, for he only counted the spat that had survived till in autumn, when already about 9 out of 10 of the spat that originally attached will have dis- appeared.

Orton (1937 a) assumes that oysterlarvae prefer to set on dark surfaces and recommends the use of dark-coloured cultch. Orton does not tell us how he came to the conclusion that dark-coloured surfaces catch more spat than white; he only mentions a small-scale application of black-varnished shells.

To investigate this matter I used glass plates frosted on both sides and coated with a coloured paste. Twelve of these plates were placed at an angle of 45° in oblong containers of reinforced concrete (fig. 19). I eliminated differences in the death-rate by applying the same methods as were used with the plates in the experiments described above. The places of the coloured plates in the container were regularly interchanged.

The coating of the plates was composed of dye, cement, lime, sand and water; the proportions of the components were modified emperically and we compared the tints obtained after the dry plates had been immersed in seawater. Several plates appeared to show a less intensive colour after immersion in seawater, some dyes even changed their colour. At last we succeeded in finding suitable compositions of the coatings, so that finally we obtained plates which showed bright colours in seawater.

We tried to avoid dyes with poisonous components, such as lead and mercury.

The coating of the white plates contained lime and fine white sand, but no cement. The yellow paste contained yellow ochre, cement and sand. All the other coatings contained sand, cement, lime and dye. I used ultramarine (blue), Swedish black, an unidentified green dye of the group of the crystal-greens and a red analine-dye of which the colouring component is "litholechtscharlach".

I obtained the following results [1]) (station Yersche Bank):

Periods of exposure	White			Red			Yellow		
	upper-side	under-side	total	upper-side	under-side	total	upper-side	under-side	total
3 VII-12 VII 1938	35	20	55	36	15	51	24	16	40
15 VII-21 VII 1938	12	6	18	9	7	16	14	10	24
21 VII-27 VII 1938	37	21	58	26	21	47	22	15	37
27 VII- 2 VIII 1938	4	4	8	10	5	15	8	5	13
2 VIII- 8 VIII 1938	59	33	92	75	33	108	65	32	97
8 VIII-14 VIII 1938	362	120	482	364	108	472	253	113	366
Totals	509	204	**713**	520	189	**709**	386	191	**577**

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[1]) For each colour I divided the total number of spat caught by the number of plates used.

Periods of exposure	Green			Blue			Black		
	upper-side	under-side	total	upper-side	under-side	total	upper-side	under-side	total
3 VII-12 VII 1938	16	7	23	40	15	55	34	21	55
15 VII-21 VII 1938	6	3	9	13	7	20	10	10	20
21 VII-27 VII 1938	17	8	25[1])	23	16	39	17	13	30
27 VII- 2 VIII 1938	6	2	8[1])	9	1	10	9	5	14
2 VIII- 8 VIII 1938	47	10	57	58	29	87	54	27	81
8 VIII-14 VIII 1938	231	54	285	353	117	470	288	96	384
Totals	323	84	**407**	496	185	**681**	412	172	**584**

The differences between the number of spat caught under field-conditions on substrata of different colours are not great. Only the number of spat on the green plates is considerably smaller than that on the plates of other colours. I am sure, however, that it is not the colour green which causes this difference, but the chemical nature of the dye. I counted the spat 6 to 9 days after attachment, just as I did the spat of other series. The spat then measured 0,6 to 0,8 mm, but the spat on the green plates showed little or no growth after attachment; it measured 0,37 mm on an average. During the periods from 21 VII to 27 VII 1938 and 27 VII to 2 VIII 1938 I used green plates that had been used once before. These plates caught about the same number of spat as the plates of the other colours and the size of the spat appeared to be about the normal. Probably the poisonous character of the dye had decreased after having been immersed for 12 days. The green dyes of the group of the crystalgreens are known to have a bacteriostatical effect, so that we were not surprised to find that it affected other living beings.

In 1939 I placed black and white plates in an container in a horizontal position. Two series of three plates in vertical order: white-black-white and black-white-black. The results are stated in the table on page 227 (station Yersche Bank).

Just as in 1938 the white plates appeared to have caught somewhat more spat than the black ones, but the difference is not great, perhaps even negligible. It is not impossible that this difference was caused by the far heavier setting of barnacles on the black plates. I also observed in 1938 that black plates

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[1]) The green plates of these series had been used once before.

Periods of exposure	Black plates									averages
	topmost			intermediate			bottommost			
	upp.	und.	tot.	upp.	und.	tot.	upp.	und.	tot.	
2 VII- 8 VII 1939	43	12	55	50	15	65	57	17	74	65
8 VII-14 VII 1939	65	17	82	41	24	65	45	14	59	69
14 VII-20 VII 1939	105	33	138	94	22	116	71	44	115	123
20 VII-26 VII 1939	10	6	16	9	7	16	11	6	17	16
26 VII- 4 VIII 1939	70	15	85	42	25	67	67	20	87	80
14 VIII-10 VIII 1939	21	18	39	23	9	32	19	29	48	40
10 VIII-16 VIII 1939	30	17	47	25	20	45	30	17	47	46
19 VIII-22 VIII 1939	27	16	43	32	10	42	35	5	40	41
Totals	371	134	**505**	316	132	**448**	335	152	**487**	**480**

Periods of exposure	White plates									averages
	topmost			intermediate			bottommost			
	upp.	und.	tot.	upp.	und.	tot.	upp.	und.	tot.	
2 VII- 8 VII 1939	50	12	62	49	11	60	52	12	64	62
8 VII-14 VII 1939	140	25	165	72	14	86	70	18	88	113
14 VII-20 VII 1939	105	15	120	142	19	161	119	31	150	144
20 VII-26 VII 1939	7	4	11	10	4	14	10	4	14	13
26 VII- 4 VIII 1939	99	18	117	88	20	108	70	19	89	105
4 VIII-10 VIII 1939	50	22	72	10	20	30	30	17	47	50
10 VIII-16 VIII 1939	46	22	68	27	5	32	29	15	44	48
19 VIII-22 VIII 1939	35	9	44	30	6	36	8	11	19	33
Totals	532	127	**659**	428	99	**527**	388	127	**515**	**568**

caught a heavier set of barnacles than white plates. This is in accordance with ZOBELL's statement (1938) that red-brown and black substrata catch more barnacles than white, yellow and blue substrata.

Summarizing I conclude that under field-conditions little or no influence of the colour of the substratum on the intensity of setting could be shown. There is no reason to recommend the application of collectors of another colour than those commonly used, as for instance, white tiles [1]).

[1]) Tile-collectors are coated with a mixture of lime and sand. This is not done to obtain a white colour, but to make it possible to detach the spat after some time ("detroquage").

It should be remembered that the water in the Oosterschelde is rather turbid and that the column of water above my container varied from 2 to 6 metres, so that it is not impossible that the influence of the colour of the substratum on the intensity of setting is greater when the plates are illuminated more intensely. Larvae of barnacles are in any case more sensitive to colour than oysterlarvae.

The influence of cleanness and roughness on the intensity of setting

Successful attachment is only possible when the surface of the substratum is clean enough to fix the cement from the byssus-gland on it. Many authors state that clean surfaces are un-doubtedly the most suitable for settlement (e.g. PETERSEN 1908, MAZZARELLI 1922, KÄNDLER 1930, GALTSOFF, PRYTHERCH and MAC MILLAN 1930, HINARD 1932, NEEDLER 1932 a, ORTON 1937 a, HOPKINS 1937).

HOPKINS (1937) tested roughly the decrease in efficiency of cultch after it had been in water for some time. Shells exposed in wire bags appeared to have lost a good deal of their efficiency as spat-collectors in 9 days. Local conditions have much influence on the rate at which the suitability of cultch-material decreases. Algal growth shows great differences at various depths and the deposition of silt is not the same everywhere. The plates of my "ordinary series", with which I ascertained the intensity of the spatfall, remained in the water for 3 days and the plates of my special series for 6 days. In the containers with which I tested the influence of the roughness of the surface on the intensity of setting I placed i.a. plates with a "normal" coating at an angle of 45° (the "normal" angle) for 6 days at the station Yersche Bank.

Periods of exposure	2 × 3 days	1 × 6 days
3 VII–12 VII 1938	48	64
15 VII–21 VII 1938	29	28
21 VII–27 VII 1938	67	41
27 VII– 2 VIII 1938	30	24
2 VIII– 8 VIII 1938	123	85
8 VIII–14 VIII 1938	610	523

A comparison of the number of spat attached to these plates with the number on the two ordinary 3-days series from the same station will show whether or not the plates have lost much of their efficiency as spat-collectors after three days (see page 228).

We may conclude from these data that, although a slight decrease in efficiency of the spat-collectors can be observed after 3 days of immersion, the plates maintain their suitability during a period of at least 6 days. After about 10 days the suitability decreases rapidly, while a growth of organisms, for instance of algae, may then be observed with the naked eye. I stated above that in the year 1938 the potential spatfall in August was enormous, but that the partical spatfall was a failure owing to the fact that the tile-collectors had been immersed many days too early.

The roughness of the substratum often appeared to influence the intensity of setting. HORST (1884) exposed in the Oosterschelde plates of smooth glass, frosted glass and limed glass. The limed glass appeared to catch far more spat than the smooth glass and the frosted glass. LEES (1930) in his attempts to invent new kinds of cultch-material found that the larvae of *Ostrea edulis* attach intensively on limed glass. HINARD (1932) stated that the larvae prefer to attach on substrata which contain lime: "Elles semblent avoir une prédilection pour les supports calcaires"; but VOISIN (1933) tells us that lime is not indispensible and that the cultch-material ought to be clean and firm in the first place. COLE (1938 a) and COLE and KNIGHT JONES (1939) tell us that it is difficult to induce larvae of *Ostrea edulis* to set on smooth glass. They caught far more spat on slates (tank-observation).

Similar observations have been made on other kinds of oysters.

Ostrea virginica

NELSON (1930) compared the setting on smooth glass, ground glass and objects (glass and shells) coated with a mixture of lime and cement. The limed objects caught far more spat than the others. PRYTHERCH (1934 a) observed that the cement coating of the partition-collectors, which is very rough, is ideal for attachment of the larvae of *Ostrea virginica*. Smooth glass slides and china placed near and inside these collectors gathered

far less spat per unit of surface than the partition-collectors. Ground glass was nearly as effective as the cement surface of the partition-collectors.

Ostrea gigas

HORI (1936) observed that the spatfall on frosted glass was poor, although mature larvae of *Ostrea gigas* were abundant at that time. YOKOTA (1936) states that it is impossible for the larvae of this kind of oyster to attach on objects coated with paraffin.

Ostrea lurida

COE and ALLAN (1937) caught far more spat of *Ostrea lurida* on wood and cement than on smooth glass. The spatfall was more intense on glass that had been used once before. They presume that it is perhaps organic matter in decomposition that brings about this difference. In connection with this I mention ZOBELL (1938), who tells us that several sessile organisms attach more intensely on objects of which the surface is covered with a film of bacteria than on sterile surfaces.

To investigate this matter I placed in a long container plates with different surfaces: smooth glass, ground glass and glass with a coating of lime, cement and sand. I used fine sand (the same as I used for my ordinary plates) as well as sand of fairly coarse and of very coarse grain. In a dry state the coating with fine sand is very smooth to the naked eye, while the other

Periods of exposure	Smooth glass	Ground glass	Smooth coating	Rough coating	Very rough coating
3 VII–12 VII 1938	1	2	64	54	60
15 VII–21 VII 1938	0	0	21	20	17
21 VII–27 VII 1938	0	1	42	38	26
27 VII– 2 VIII 1938	0	3	24	30	35
2 VIII– 8 VIII 1938	3	13	85	90	95
8 VIII–14 VIII 1938	18	101	523	585	690
Totals	22	120	759	817	923

coatings are respectively rather and very rough. I placed two or three plates of the same kind in the container and regularly interchanged the places of the different materials. The results obtained are stated in the table on page 230 (station Yersche Bank).

The coated plates appeared to catch far more spat than the plates of smooth glass and frosted glass. Smooth glass is very unsuitable for attachment of *Ostrea edulis*. The numbers of spat caught on the coated plates of different roughness did not diverge very much. Consequently macroscopical roughness is probably not essential to attachment. These small differences may probably be ascribed to the enlargement of the surface owing to roughness.

Although the coating of the ordinary plates looks smoother than ground glass, the former proved to be more suitable as spat-collector. In fact the surface of the coating is much rougher than that of ground glass, as the microscope will show. I dare not yet decide whether the chemical composition of the substratum may not play some part, but it is not impossible that it is merely the microscopical roughness which brings about the difference in the intensity of setting discussed above. The larvae seem to require or at least to prefer microscopical roughness. Perhaps it is not only the suitability for fixation that plays a part, but also the suitability for crawling, for it is possible that the larvae are more easily washed off from one kind of substratum than from another.

SUMMARY

1. A crisis in oyster-culture in the years following 1930, caused by an extremely rapid propagation of the slipper-limpet and an aggravation of shell-disease compelled Dutch oyster-farmers to abandon spat-collection on sown-out shells and to revert to tile-collectors. They were assisted by the Government in various ways, one of which was the prediction of the time of setting, by which the chances of a good spatfall on the tiles are increased.

2. The water in the basin of the Oosterschelde performs an almost perfectly oscillating movement, which has a favourable influence on the water-temperature and at the same time guarantees a satisfactory retention of the larvae.

3. The Oosterschelde has a rather high and constant salinity.

The fluctuations in salinity are correlative with fluctuations in the discharge of the big rivers. Local rainfall has no perceptible influence on the salinity of the Oosterschelde.

4. Owing to the great tidal range the water in the basin is regularly mixed very thoroughly, which prevents stratification.

5. At high water the basin contains 675.000.000 m³, at low water 275.000.000 m³. At each tide about 3,7 % of the water of the basin (25.000.000 m³) is replaced by water from the districts situated farther west.

6. At several stations the current-velocity in the surface layers attains 100 to 150 cm/sec. at the height of the tide; about 50 cm/sec. in shallow places. Near the bottom the velocity is about 1/3 less.

7. Practically every summer the water-temperature rises above 18° C. for a considerable period of time. Temperatures above 22° C. occur only sporadically. Owing to the thorough mixing of the basinwater the daily fluctuations in water-temperature are small.

8. The Dutch methods for the numerical determination of oysterlarvae have been modelled on the French methods, but important modifications have been introduced, so that the Dutch plankton-samples are undoubtedly better quantitative than the French.

9. In the course of the summer season plankton-samples are procured daily, under comparable circumstances, from 100 litres of water at two stations in the Oosterschelde. The one station is situated in the centre of larvae-production (Yersche Bank), the other in an important tile-centre (Kattendijke). All the larvae in the samples are counted and measured.

10. In consequence of the frequent sex-change the spawning of *Ostrea edulis* is spread over a greater number of days than that of non-incubatory oysters.

11. As our knowledge of the influence of temperature and nutrition on sex-change is as yet insufficient, we are unable to predict the way in which egg-production will be distributed over the season. In practice it is of less importance to know the moment of the beginning of spawning than the date at which spawning reaches its height.

12. Spawning in August may probably be largely ascribed to oysters that pass through the female stage a second time.

13. As egg-maturation requires a certain amount of warmth,

it is not possible to fix a clearly defined limit below which spawning does not take place.

14. The intensity of spawning decreases as the season advances. It is not possible to indicate the exact limit at which in autumn spawning ceases altogether. Some oysters bearing larvae may still be found long after the temperature has fallen below 18° C.

15. Although there are indications that spawning attains its greatest maxima at the spring-tides, we are not justified in saying that spawning preferably shows its greatest maxima at full moon.

16. There cannot be a strict parallelism between the periodicity of spawning and the actual water-temperature, as *Ostrea edulis*, owing to frequent sex-change, does not show a simultaneous occurrence of ripe eggs in a great part of the stock.

17. Although the water-temperature influences the duration of incubation, we cannot expect the periodicity in swarming to run strictly parallel to the course of the actual water-temperature, as the intensity of swarming is entirely dependent on the intensity of the spawning preceding it.

18. The larvae are liberated after having been sufficiently incubated. The mother oysters do not await favourable weather-conditions for this. Unfortunately a considerable part of the larvae will therefore be found to swarm under unfavourable circumstances.

19. In the centre of larvae-production liberation causes sharp increases in the number of larvae. These increases are mostly followed by sharp drops, which, however, are not a sign of a great mortality of the larvae, but are a consequence of the dispersion of the larvae through a greater volume of water.

20. The annual extent of larvae-production is determined by the number of mature oysters on the banks, the ages of these oysters, the age at which the oyster reaches sexual maturity in the district concerned, the percentage of oysters participating in female reproduction every year and the number of larvae produced by one oyster.

21. Female reproduction in the second summer may occur in the Oosterschelde, but is of little practical importance.

22. In favourable summers probably about 100 % of the population takes part in female reproduction. Many oysters probably produce larvae twice a year.

23. Larvae-production in the Oosterschelde steadily increased

from 1936 till 1939 owing to an increase in the number of adult oysters.

24. Oysterlarvae at swarming measure 0,165 to 0,200 mm, the majority 0,175 to 0,185 mm. Temperature is not all-powerfull in regulating the size of the swarming larvae. The size of the newly-liberated larvae tends to decrease towards the end of the season. Full-grown larvae measure 0,260 to 0,300 mm, in most cases 0,275 to 0,285 mm.

25. The vertical distribution of the oysterlarvae in the Oosterschelde is essentially the same in daylight and at darkness.

26. In the Oosterschelde temperature and salinity have no influence on the vertical distribution of the larvae, as strong tidal currents prevent all stratification of the water here.

27. The vertical distribution of the oysterlarvae in the Oosterschelde is essentially the same in stormy weather with rough sea and in calm weather with smooth water.

28. Although the current-velocities at many stations exceed 50 cm/sec during a great part of the tidal cycle, it must not be thought that at these velocities the larvae drop to the bottom.

29. During strong currents the larvae of the water-layers near the bottom are not passively swept to the surface layers.

30. As the oysterlarvae do not drop to the bottom at any stage of the tide or of a space of 24 hours, they are quite at the mercy of the tidal streams. The greater the distance between a given station and the basin, the shorter the period during which the basinwater and with it a large number of larvae will be present at that station.

31. Owing to the proximity of liberating adults, the course of the number of larvae is less even in the centre of larvae-production than at other stations. The number of older larvae, however, does show an even course here.

32. Water-temperature has a very great influence on the duration of the pelagic stage. The differences in salinity are too small here to influence the duration of the pelagic stage. Any protraction of the pelagic stage decreases the percentage of the larvae that reach the full-grown stage.

33. As regards the Oosterschelde only about one third of the losses during the pelagic stage is attributable to the tidal water-renewal.

34. Wholesale dropping to the bottom of full-grown larvae is out of question.

35. The spat prefers to attach with the umbo pointing in a special direction. The pull of gravity is the orienting factor in this.

36. At several stations here the intensity of the potential spatfall is measured quantitatively in the course of the season of reproduction.

37. It is possible to demonstrate a distinct correlation between the number of mature larvae and the intensity of spatfall. The proportion between the number of full-grown larvae and the intensity of spatfall is different for each station, but constant for one station.

38. Less than 10 % of the larvae reach maturity; about 1 à 2 % of the mature larvae succeed in attaching; 90 % of the newly-set spat already dies before winter. About 250 out of every 1.000.000 produced larvae attach.

39. The oysterfarmer by selecting the right place and time for the planting of his collectors should try to raise the percentage of mature larvae accomplishing fixation to the highest possible degree.

40. Environmental conditions, such as temperature, salinity and copper-content have little direct influence on the process of fixation in the Oosterschelde.

41. In considering the correlation between swarming and setting it should be known what has become of the pelagic larvae.

42. Prediction of time and intensity of setting at short notice has proved to be very well possible here and is practised with success.

43. It has been shown that light has no perceptible influence on the fixation process under field-conditions. It is, however, possible that oysterlarvae may show a predilection for shaded situations in case the intensity of light at fixation happens to be great.

44. The current may have a favourable influence on the spatfall by providing a regular supply of larvae, but also an unfavourable influence, by washing off the crawling larvae from the substratum. Places much exposed to currents are consequently less suitable for fixation.

45. Most of the spat settles roundabout still water in consequence of the influence of the current on the setting process. The number of larvae in the course of the tide determines whether at a given station the intensity of setting will be greatest at high water or at low water.

46.   In the Oosterschelde more spat settles near the bottom than near the surface. This is not attributable to differences in the vertical distribution of the full-grown larvae, but to differences in current-velocity.

47.   The suitability of a place for spatfall is mainly determined by the number of mature larvae present in the course of the tidal cycle and by the course of the current-velocity. Other factors determine what percentage of the settled spat shall survive.

48.   Under natural conditions more spat settles on upper-surfaces than under-surfaces in the Oosterschelde, vertical surfaces being the least suitable for attachment.

49.   Under field-conditions the colour of the substratum has little influence on the intensity of setting.

50.   The degree of roughness of the substratum has a great influence on the intensity of setting. Microscopical roughness is much more important than macroscopical roughness.

Practical applications

A.   In consequence of its special hydrographical conditions the Oosterschelde is no doubt very suitable for the production of oyster-spat.

B.   Owing to differences in watertemperature and in the course of the production of larvae the setting-maxima do not occur in the same part of the season every year.

C.   By far the best results are obtained with collectors when they are placed just at the moment that a large setting maximum is to be expected.

D.   A great setting-maximum may be expected when a considerable production of larvae occurs at a high water-temperature. At water-temperatures below 18° C. no spatfall of any importance is to be expected, even though the supply of larvae should be considerable.

E.   Prediction of the spatfall at long notice is not possible in Holland, owing to the fitful weatherconditions and the intricacy of the problem of the periodicity in larvae-production.

F.   Prediction of the spatfall at short notice is very well possible. Besides registration of the water-temperature this requires frequent determinations of the number and size of the

oysterlarvae per volume of water. Care should be taken that the plankton-samples are quite comparable.

G. The best spatfall may be expected in places where the number of full-grown larvae is large at the moment that the current-velocities are small. These factors are largely governed by the tidal movements.

H. Collectors can be placed close together in large numbers without any objection, if only care is taken that the masses are not too compact, as then they would not be sufficiently accessible for the larvae-bearing water.

I. Near the bottom the current is less unfavourable for fixation than near the surface. Consequently there is no reason to recommend spat-collection by means of floating collectors. The number of mature larvae is about the same at any depth.

J. The results of the inquiries into the influence of the angle of surface on the intensity of setting have shown that there is no reason for the construction of special types of collectors. Upper-surfaces catch more spat than other surfaces, but at the same time the former catch more silt, so that more spat is smothered on them. Cemented cardboard collectors cannot be used here, as wave-action and currents would soon smash them to pieces.

K. There is no reason why collectors should be protected against light, as in practice there is little evidence of a difference in setting in light and in dark places.

L. There is no reason why another colour than the usual should be used for collectors.

M. The roughness of surface of the collectors commonly used here is highly conducive to fixation.

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NON-MARINE MOLLUSCA FROM THE SATELLITE ISLANDS SURROUNDING JAVA

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With 2 Maps and 4 Figures. [1])

I. INTRODUCTION

In recent years various Dutch naturalists and a few other travellers paid occasional visits to one or more of the numerous little islands off the coast of Java and succeeded in securing quite a satisfactory miscellany of non-marine mollusca. Indeed, however much these spoils are casual and the observations discontinuous, yet by their combined action it became possible to draw up faunules of the non-marine molluscs of the respective insular outposts of Java, a proceeding which had never been attempted so far.

Only in a few cases the travellers were in pursuit of special investigations. Thus Dr. K. W. DAMMERMAN, at that time Director of the Zoological Museum of Buitenzorg (Java), visited several of the islands in question, studying the soil and surface fauna; the same author, in collaboration with Prof. Dr. W. M. DOCTERS VAN LEEUWEN, at that time Director of Botanic Gardens, Buitenzorg, carried out investigations on the repopulation of Krakatau, after the catastrophal eruption of 1883; M. A. LIEFTINCK, at that time Curator of the Buitenzorg Zoo-

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[1]) The author of the paper and the Editor of the Archives Néerlandaises de Zoologie are very much indebted to the Trustees of the Zoölogisch Insulinde Fonds for a grant by which the publication was made possible.

logical Museum, visited the Karimon Djawa Islands on behalf of an entire zoological survey of this group. For the rest we have to do with incidental observations only, leaving ample opportunity for future research.

The number of species which I could identify, resp. check, amounted to 116. Partly they belong to the Amsterdam Zoological Museum (A.), partly to the Buitenzorg Zoological Museum (B.) and partly to the Rijksmuseum van Natuurlijke Historie at Leiden (C.). For the allowance to include the data belonging to the two last-named institutions I am very much indebted to Messrs. M. A. LIEFTINCK, at present Director of the Buitenzorg Museum, and to Dr. CH. BAYER, Curator of the Leiden Museum respectively.

Next I included all the references in literature alluding to the occurrence of non-marine mollusca in one of the islands under consideration. In this way the total amount of species augmented to 127, not counting the varieties in such cases where the main form is also represented. This sum of 127 species will be treated in an annotated list in systematic sequence in Paragraph III. At each entry is mentioned: 1. the original diagnosis, and 2. such other systematic literature as is indispensible for a proper understanding of the species.

Besides I tried to check the general distribution of each of the species, especially whether or not they are found in Java.

The islands which underlie the present paper, 36 in all, are very different in size, geomorphology, geology, climate, vegetation and other factors. Therefore they are difficult to compare. In fact their mutual connections are often minimal, presenting no other causal relation than their joint dependence on the principal island, Java.

In Paragraph II I will give a list of all the islands, together with some information in regard to orography, structure, climate and organic life. They are enumerated in a circle around Java, beginning at the South West point, thence continuing via West-, North-, East- and South-coast, ending likewise in the South West. The exact position of each island can be ascertained on the accompanying sketchmaps (Map I and II).

The systematic part is ensued by a list in which the fauna of each individual island is summarized. This Paragraph IV allows us to compare the faunules of the islands reciprocally and with Java.
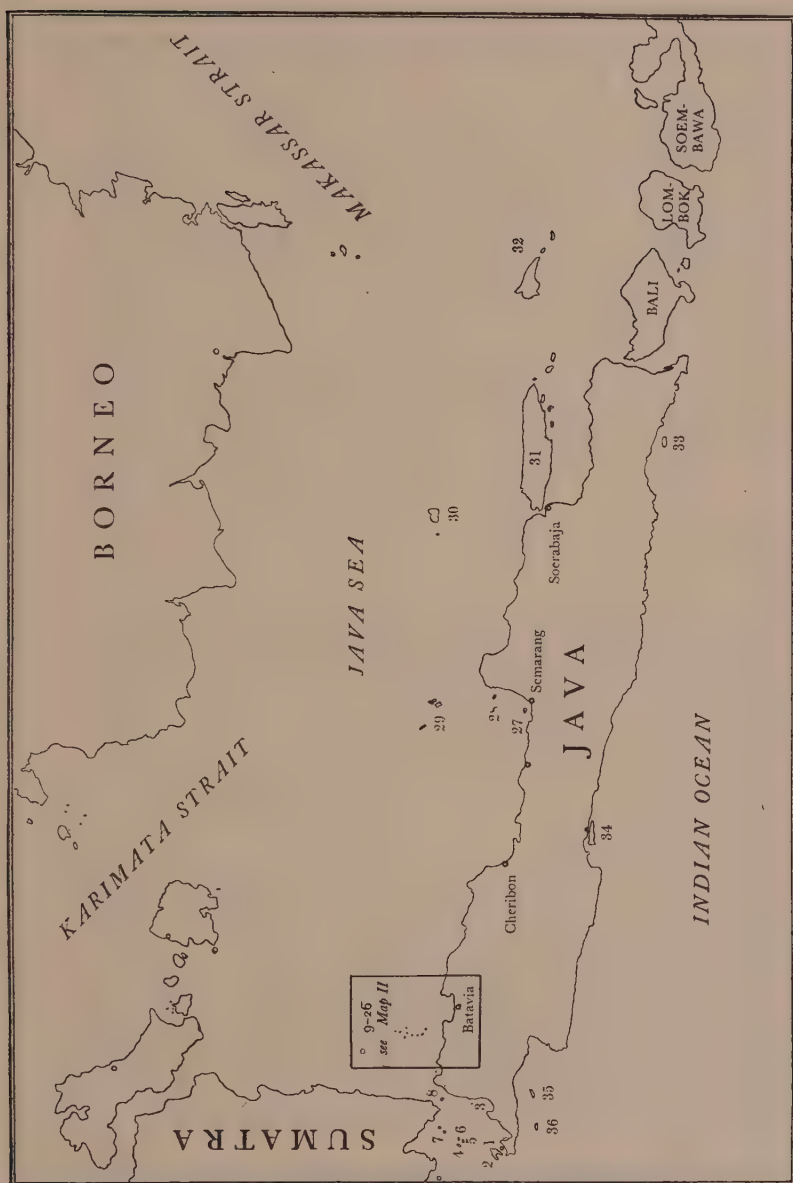
Map I.   The Island of Java with its Satellites. (The framed territory of the Duizend Islands is enlarged in Map II).
1. Meeuwen Island; 2. Prinsen Island; 3. Popole Island; 4. Krakatau Island; 5. Verlaten Island; 6. Lang Island;
7. Sebesi Island;   8. Dwars in den Weg Island;   9–26. Duizend Islands (see Map II);   27. Small Island near
Karang Anjer; 28. Poeloe Pandjang; 29. Karimon Djawa Islands; 30. Bawean Island;   31. Madoera Island;
32. Kangean Islands; 33. Noesa Baron; 34. Noesa Kembangan; 35. Trouwers Island; 36. Klapper Island.

II. SHORT DESCRIPTION OF THE ISLANDS

The majority of the islands treated in this paper are either very small and unimportant, or lying so far out of the reach of European interest, that they are hardly known to the general public. Therefore it seems not superfluous to give here in short some general information on position, configuration, physiographical conditions, vegetation and animal life of each of the 36 islands where non-marine mollusca have been collected.

This information was partly drawn from encyclopedia's and general treatises, partly from special literature of a limited area.

The general works are: De Zeemansgids voor Nederlandsch-Oost-Indië, 6-th edition, Westelijk gedeelte (1929) and Midden gedeelte (1931), the Encyclopedie van Nederlandsch-Indië, various articles in: De Zeeën van Nederlandsch Oost-Indië, 1922, and in: Science in the Netherlands East Indies, 1929.

For general geological survey I had the fundamental help of R. D. M. VERBEEK & R. FENNEMA, Geologische Beschrijving van Java en Madoera, Vol. 1, 1896 and of L. M. R. RUTTEN, Voordrachten over de Geologie van Nederlandsch Oost-Indië, 1927.

Biological data can be obtained from L. F. DE BEAUFORT, Zoögeographie van den Indischen Archipel, 1926, B. RENSCH, Die Geschichte des Sundabogens, 1936 and Drie Jaren Indisch Natuurleven, 11-de Verslag (1936-1938) van de Nederl. Indische Vereeniging tot Natuurbescherming, 1939.

The other literature, mostly articles of limited scope, treating one, or a few islands in particular, will be mentioned at the entries of the respective islands.

1. *Meeuwen Island*. Lying in Meeuwen Bay, off the SW-coast of Java, opposite the peninsula Java's Eerste Punt (Java's First Point). It belongs to the Residency of Bantam. Occupies circa 450 ha, almost flat country, the highest point not exceeding 78 m alt. The island is partly covered with forest, in which wildboar, deer and a small chevrotain find shelter, partly with a sort of parkwood, a light wood with fine views. It is uninhabited, only native fishermen calling now and then during their cruises. Meeuwen Island is a game reserve since 1921 (extended 1937).

Literature: ANON., Het schiereiland Djoengkoelon. Tijdschr. Binnenl. Best. Vol. 39, 1910, p. 136-140.

2. *Prinsen Island*. Large island off Java's Eerste Punt (Java's First Point) separated from Java by the Prinsen Strait or Behouden Passage, a deep passage, 8 km across, allowing large sea-vessels to anchor close to the shore. It belongs to the Residency of Bantam.

The surface of the island covers 17.000 bahoe or 12.000 ha, partly mountainous, partly low country and swampy land. The highest summit, Goenoeng Raksa, is 320 m. The island consists of igneous rock (pyroxene andesite). It is horse-shoe-shaped, the emerging part being the remnants of a very large caldeira with a radius of 5.65 km. On the SW the wall is interrupted, and here the sea enters a wide and very exposed bay, Casuaris Bay.

The island has a luxuriant vegetation of virgin forest, especially along the W-coast, where it is inhabited by tigers, wild deer and wildboar. It is uninhabited, only native fishermen calling now and then during their cruises. The entire island is a game reserve since 1921 (extended 1937).

Literature: H. TH. KAL, Prinseneiland. Tijdschr. Binnenl. Best. Vol. 39, 1910, p. 76–78.
H. A. ELIAS, Met de Laurens Pit naar Prinseneiland en Djoengkoelon. Tijdschr. Binnenl. Best. Vol. 39, 1910, p. 186–203.
R. A. EEKHOUT, Prinseneiland en het Schiereiland Djoengkoelon. Tijdschr. Binnenl. Best. Vol. 39, 1910, p. 275–305; also in: Ind. Mercuur, 1911, p. 66.
W. C. A. VINK, Rapport omtrent het onderzoek naar den vischrijkdom der wateren rondom Djoengkoelon en Prinseneiland. Meded. Vissch. Stat. no. VI, 1911, p. 44–52.

3. *Popole Island*. Lying in the Peper Bay, close to the Bantam West-coast. It is a low, sandy island, surrounded by coral reefs, belonging to the Residency of Bantam. Very little is known about it, not even the exact size.

4, 5, 6. *Krakatau Island, Verlaten Island* and *Lang Island*. These three, together forming the Krakatau group, are lying on the edge of a caldeira, with a diameter of circa 7 km, in the middle of Soenda Strait. The distance to Prinsen Island and to Java's Derde Punt (Java's Third Point) is about 40 km, to Sebesi Id. 12 km and to the Sumatra coast some 40 km. All three belong to the Residency of Lampongsche Districten (Sumatra).

The elevation of Krakatau is 813 m, of Verlaten Id. 182 m and of Lang Id. 172 m. The islands consist of igneous rock, pyroxene

andesite occurring. The two first are not inhabited. Krakatau and Verlaten Id. were proclaimed a nature reserve in 1929, together they cover circa 2500 ha. (Krakatau 1400 ha, Verlaten Id. 1100 ha.). Lang Id. was not included in this decree as it is inhabited now and then, and therefore does not warrant an undisturbed flora and fauna.

In historical times the configuration of the islands of the Krakatau group underwent several modifications by earthquakes and eruptions. By far the best informed we are on the severe cataclysm of August 26-28, 1883. This eruption, with its entire aftermath of meteorological, geological and biological events, has acquired world-wide fame. The history of the animal re-population and the re-afforestation of the islands during the last half century has been the subject of a great number of interesting publications, far too numerous to find room here.

At the present day all three islands, which were entirely sterelized during the great eruption, are covered again with a continuous vegetation, which, although luxuriant and varie-gated, is not the normal jungle vegetation as we find in the neighbouring parts of Java or Sumatra. An analysis of the Krakatau flora and its plant associations fully demonstrates that we have to do here with an unstable vegetation which is still in constant evolution, in so far as certain species are dominating and others are lacking altogether, the proportionate numbers fluctuating from year to year.

The fauna, especially that of Krakatau proper, which is the best studied, is not yet normal either, as rats, mice and a few bats are the only Mammals. Reptiles are very scarce, frogs and toads, and freshwater fishes are entirely absent, not to speak of other animal orders.

Of the Molluscs which were found in Krakatau before the eruption (*Cyclophorus perdix*, *Hemiplecta bataviana*, *H. javacensis* *Chloritis helicinoides* and *Amphidromus inversus*) none is found at the present day. What we see now is half a dozen of moss-fauna snails, a few forms from the Barringtonia-, or Casuarina-community and one real tree-snail: *Amphidromus porcellanus*. The affinities of all these are without reserve pointing to Java.

Literature: R. D. M. VERBEEK, Krakatau. Vol. 1, 1884, Vol. 2, 1885.
De Opneming van de Krakatau-Groep in Mei 1908. Jaarversl. Topogr. Dienst, 1908, (1909).

K. W. Dammerman, The Fauna of Krakatau, Verlaten Island and Sebesy. Treubia, Vol. 3, 1922, p. 61–112.

Ch. E. Stehn, W. M. Docters van Leeuwen & K. W. Dammerman, Krakatau. Fourth Pacific Science Congress, 1929.

A. Ernst, Das biologische Krakatauproblem. Viertelj. Schr. naturf. Ges. Zürich, Vol. 79, 1934, p. 1–187.

W. M. Docters van Leeuwen, Krakatau, 1883–1933, A. Botany. Ann. Jard. Bot. Buitenzorg, Vol. 46–47, 1936, 506 pp. 36 pl. 1 map.

7. *Sebesi Island*. Is a small volcanic island not far from the Sumatra coast (distance circa 15 km) and almost right north of the Krakatau group (distance about 19 km). It belongs to the Residency of Lampongsche Districten (Sumatra). The highest elevation is 844 m. The size in m. square is unknown. The island is almost entirely clad with virgin forest. Although it must have suffered a great deal from the Krakatau eruption of 1883, organic life was not totally destroyed, a fact which is nowadays still discernible in its vegetation, which is much more normal and not so rapidly modifying as that of Krakatau. Since 1890 it is again inhabited.

Literature: K. W. Dammerman, The Fauna of Krakatau, Verlaten Island and Sebesy. Treubia, Vol. 3, 1922, p. 61–112.

W. M. Docters van Leeuwen, The Vegetation of the Island of Sebesy, situated in the Sunda Strait, near the Islands of the Krakatau-group; in the year 1921. Ann. Jard. Bot. Buitenzorg, Vol. 32, 1922, p. 135–192.

8. *Dwars in den Weg Island*. In the narrowest part of Soenda Strait, at 10 km distance off the Bantam coast and belonging to the Residency of Bantam. It consists of several small islands, surrounded by coral reefs. The NE part is a caldeira wall with a radius of 1.2 km. The group is composed of igneous rock (pyroxene andesite). Total surface 7.34 km. square. Highest point 155 m. Dwars in den Weg is thickly covered with virgin forest and uninhabited.

9. *Duizend Islands*. (Map II). A group of circa 100 small, coralligenous islands N. of West Java, stretching from the Islands of Noordwachter (5°5′ South, 106°27′ East) to the Bay of Batavia and even into the latter. For convenience's sake they can be divided into 2 sections: a seaward section, being the Duizend Islands sensu stricto, and a landward section, separated from the former by a line between the Agnieten Islands and the Kombuis Islands. This landward section, although positively belonging to the Duizend Islands Archipelago, are generally

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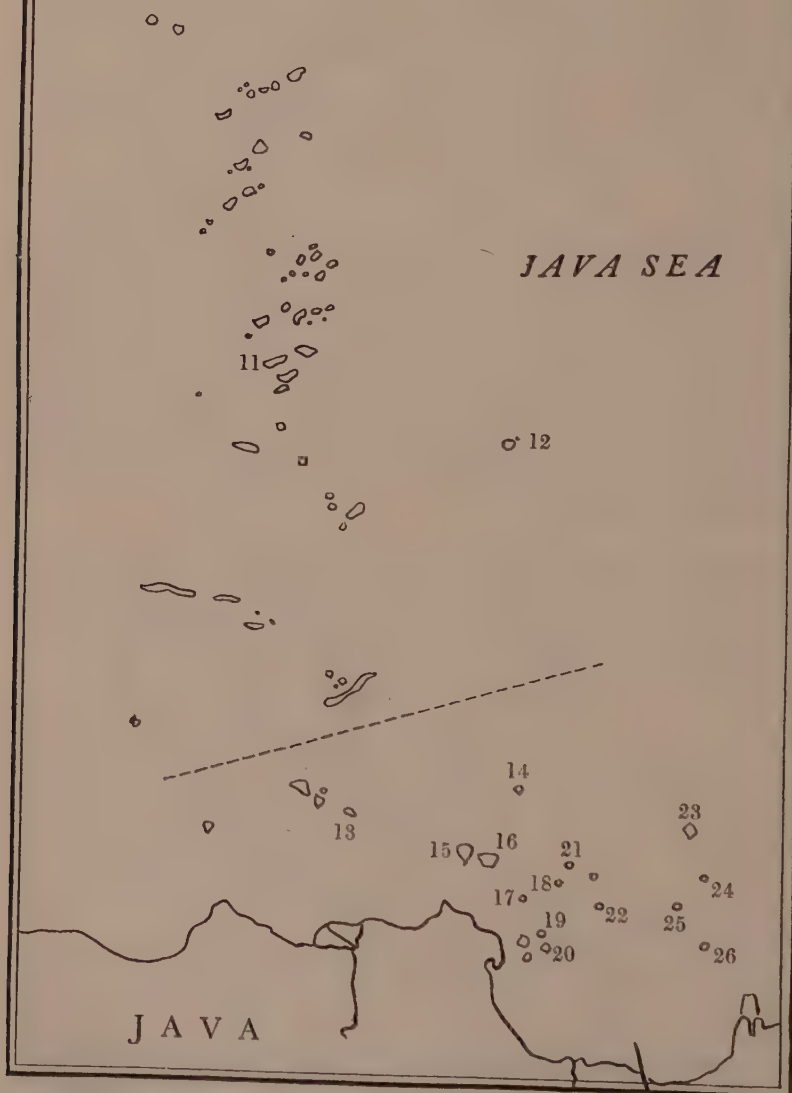
Map II. The Archipelago of the Duizend Islands. (Enlarged from Map I).
- - - is the boundary between the seaward and the landward section.
9. Duizend Islands (entire group); 10. Noordwachter Island; 11. Poeloe
Klappa; 12. Zuidwachter Island; 13. Klein Kombuis Island; 14. Dapoer
Island; 15. Middelburg Island; 16. Amsterdam Island; 17. Schiedam Island;
18. Rotterdam Island; 19. Kerkhof Island; 20. Purmerend Island; 21. Haar-
lem Island; 22. Hoorn Island; 23. Edam Island; 24. Alkmaar Island;
25. Enkhuizen Island; 26. Leiden Island.

called: Islands in the Bay of Batavia. Several of these: Alkmaar, Amsterdam, Edam, Hoorn, Enkhuizen, Purmerend, Rotterdam, Schiedam, Middelburg etc. bear the names of Dutch towns. These names were given by the old sea-farers of the 17-th century after the towns in the mother-country where "Chambers" of the Dutch East India Cy. were housed.

A very striking difference between the seaward and the landward section of the Duizend Islands is apparent in the fact that coconut trees, so common in the islands of the seaward section, are entirely wanting in all the landward islands with exception of small plantations in Edam and Haarlem.

All the islands are developed as so-called patch-reefs. Those which are sufficiently elevated above the sea are more or less densely clad with jungle and mangrove. Only few islands are inhabited, but the others are regularly visited by native fishermen. Other islands are bare reefs, sometimes hardly emerging, without any accomodation for non-marine organisms. Geologically speaking the islands are rather young constructions, as they are all dating from the Pleistocene age. They belong to the Residency of Batavia. The islands where collecting has been done are enumerated hereafter:

10. *Noordwachter Island.* Although one of the Duizend Islands, Noordwachter lies about 30 km to the north of the other islands of the group at 106°27' E and 5°5' S. It bears a vegetation of high trees, generally visible from a distance of about 14 seamiles, and is surrounded by an important fringing reef. The island bears a small lighthouse. On the NW-coast there is a little harbour with a landing stage. It has a few inhabitants.

11. *Poeloe Klappa.* At 60 km distance N.N.W. from Batavia. It is a very small island with about 600 inhabitants who are living from fishery and coconut planting. Of the original vegetation there is not much left.

12. *Zuidwachter Island.* Like Noordwachter this island lying at 106°46' E and 5°42' S, is clad with high trees, Casuarina trees along the shore and virgin forest towards the centre.

13. *Klein Kombuis Island.* It is a lowlying island, but the high trees growing on it make the island well visible from a distance of 12 seamiles. The wood is inhabited by an unusually large number of pigeons. The island is a nature reserve since 1921. It occupies an area of 18 ha. The distance to the Java coast is 8 km as the crow flies.

14. *Dapoer Island*. The island of Dapoer, lying halfway between the Agnieten Islands and Edam has a low sandy soil on a coralline nucleus and is covered for a great part with grass wildernesses and a few bushes, mixed with some solitary high trees.

15. *Middelburg Island*. Is a rather flat island, like Klein Kombuis and Amsterdam. The centre is covered with a thick, high forest, towards the periphery there is a zone of mangrove. The island is a nature reserve since 1939, especially for the protection of a colony of white ibisses, together with some species of herons and cormorans.

16. *Amsterdam Island*. Lying 5 km off the Java coast, it is of similar appearance as the preceding islands. It is clad with high trees, though not so high as in Edam. Large patches are covered with grass and rushes. Breeding colonies of ibisses, herons and cormorans are equally found in this island.

17. *Schiedam Island*. Is a bare, sandy, sickleshaped island, surrounded by a large reef.

18. *Rotterdam Island*. High trees occur in some abundance, but the original vegetation is severely threatened by the inhabitants of a kampong of native fishermen. In the N.E. part there is a zone of mangrove shrubbery.

19. *Kerkhof Island*. Is one of the few inhabited islands in the Duizend Archipelago. On the N. side are the remnants of a large fortification, a high circular wall. Close to it is an old churchyard below high trees. For the rest the vegetation bears the signs of human habitation. On the N. and E. side there is an extensive coral reef.

20. *Purmerend Island*. Is an almost circular island with a triangular prolongation on the NE side. This corner is covered with thick mangrove vegetation.

21. *Haarlem Island*. On the N-coast there is a broad sandy beach, and beyond it is a rather broad lagoon with some solitary trees. The centre is occupied by trees and shrubs.

22. *Hoorn Island*. Clad with high vegetation of trees and undergrowth. Along the N-coast there is a shallow lagoon and beyond it an extensive coral reef. Hoorn is inhabited by a colony of monkeys (*Macaca irus*), being the progeny of some 40 individuals imported in the nineties by Messrs. SELENKA and SLUITER.

23. *Edam Island.* It lies on the direct route of all mail-steamers Singapore-Java at circa 8 seamiles from Tandjong Priok. The W.side bears a 56 m high, white lighthouse and a small landing stage. The beach is narrow on this side. Behind the dwelling-place of the lighthouse guard there begins a rather thick forest. A zone of mangrove bush borders the N. and NE-coast. The island is surrounded by a broad reef. Along the N.side there is a narrow lagoon. Height of the island above sealevel circa 2 m.

24. *Alkmaar Island.* Entirely covered with low bushes. The coral shingle wall touches the N. coast of the island directly, without leaving room for a lagoon.

25. *Enkhuizen Island.* This island is a good acquaintance of all sailors by the characteristic solitary tree (Sonneratia alba) off the N.side of the reef. For the rest Enkhuizen is not very different from the other islands in the Duizend Archipelago.

26. *Leiden Island.* Is nearly entirely clad with bushes and higher trees. It occupies about 3 ha, rising only circa 1 m above highwater mark. On the N.side a shallow lagoon penetrates rather deep into the island. Along this lagoon there is a rather luxuriant vegetation of mangrove trees. The other plants are ordinary beach- and sand-dwellers. It is uninhabited, but native fishermen are calling now and then in search of firewood.

Literature: J. J. SMITH, Een tochtje naar de koraal-eilanden ten N. van Tandjong Priok. Teysmannia, Vol. 10, 1899, p. 85–95.

H. SELLEGER, De Duizend Eilanden. Tijdschr. Binnenl. Bestuur, Vol. 30, 1906, p. 414–430.

J. J. SMITH, Een botanische reis naar de Duizend Eilanden. Teysmannia, Vol. 18, 1907, p. 450–456.

J. C. KONINGSBERGER, Eenige aanteekeningen over de fauna der Duizend Eilanden. Teysmannia, Vol. 19, 1908, p. 363–374.

LANDROT, Een excursie naar de Duizend Eilanden. Tropische Natuur, Vol. 9, 1920, p. 141–148.

W. M. DOCTERS VAN LEEUWEN, Blumen und Insekten auf einer kleinen Korallen-Insel. Ann. Jard. Bot. Buitenzorg, Vol. 37, 1927, p. 1–31.

J. H. F. UMBGROVE, De Koraalriffen in de Baai van Batavia. Wetensch. Meded. No. 7, Dienst Mijnb. Ned. Indië, 1928, 68 pp., 33 pl.

————, De Koraalriffen der Duizend-Eilanden (Java-Zee). Wetensch. Meded. No. 12, Dienst Mijnb. Ned. Indië, 1929, 47 pp., 6 pl.

———— & J. VERWEY, The coral reefs in the Bay of Batavia. Excursion Guide 4th Pacific Science Congr. 1929, 30 pp., 3 pl.

C. G. G. J. VAN STEENIS, Schets van de Flora van het Eiland Dapoer. Tropische Natuur, Vol. 24, 1935, p. 31–34.

J. D. F. HARDENBERG, De Koraaleilanden in de Baai van Batavia. Drie Jaren Indisch Natuurleven, 11de Jaarversl. (1936–1938) 1939, p. 234–241.

27. *Small Island near Karang Anjer*. Nothing is known about its structure and origin.

28. *Poeloe Pandjang*. Is a low coral island, with a sandy beach on the SE point. The vegetation is for a great part composed of coconut trees with sparse undergrowth.

29. *Karimon Djawa Islands*. This group consists of circa 25 islands, large and small, situated between $100°$ and $110\frac{3}{4}°$ E and $5\frac{1}{2}°$ and $6°$ S, at nearly 9 geographical miles from the N coast of Java. They belong to the Residency of Japara-Rembang. All the islands together occupy 5833 bahoe or circa 53 km square. Of these the largest island Poeloe Karimon Djawa (or Karimon Djawa besar) measures 2567 bahoe, Poeloe Kemoedjan, the next in size, 1450 bahoe. Most of the islands are low, only P. Karimon Djawa rising to 506 m alt. The islands Parang, Njamoek, Kembar and Katang are old volcanoes.

Pretertiary rock, rich in quartz, forms the subsoil of most of the islands; basaltic intrusions, however, occurring in those which are of volcanic origin. Their geological development is probably connected more with the islands of Bangka and Billiton and with the Malay Peninsula than with Java (cfr. RUTTEN, 1927, p. 182–183).

Most of the islands are covered by coconut plantations, only the principal island, Karimon Djawa, bears some wood. The larger islands: Karimon Djawa, Kemoedjan, Mendjangan besar and Mendjangan ketjil, are inhabited by deer (*Cervus hippelaphus*). A monkey (*Macaca irus*) occurs on Karimon Djawa and Kemoedjan. In the same islands hedgehogs (*Acanthion javanica*) are common. Besides the mammalian fauna contains some rats and bats. The archipelago is especially notorious for the large number of poisonous snakes.

Freshwater is rare or absent in most of the islands. Only in the larger ones there are sparse rivulets and irrigated rice fields (sawahs). The entire population amounts to circa 1200 inhabitants, all natives, for the greater part concentrated in Karimon Djawa and a few other large islands. Of the minor islands several are uninhabited.

Literature: S. H. KOORDERS, Verslag van eene dienstreis naar de Karimon Djawa eilanden. Natuurk. Tijdschr. Ned. Indië, Vol. 48, 1889, p. 20–132.
G. L. GONGGRIJP, De Karimoen Djawa-eilanden. Historisch Economische schets. Kolon. Tijdschr. Vol. 4, 1st Part, 1915, p. 313–341, 480–497, 610–633.

30. *Bawean Island.* Situated between 5°43′ and 5°52′ S and 112°34′ and 112°44′ E, at 20 geographical miles N of Soerabaja. It belongs to the Residency of Soerabaja.

The island occupies 3.6 geographical miles square or 19931 ha. Geologically speaking Bawean is rather young, being for the greater part a volcano, now extinct, consisting of leucite and nephelian rock. Only locally some tertiary deposits, of marine origin, have been recorded. The highest summit, Goenoeng besar, reaches till 655 m. By secondary eruptions of minor importance a few small eruption-points originated on the flanks and towards the top of the old volcano, giving the mountain a very irregular, cloven appearance. At about half the height, close to the N-coast, there is a large crater lake, Telaga Kastobo, measuring 600 × 400 m across and 139 m deep, and steeply sloping down.

Rainfall is frequent and abundant during the West monsoon. Hence the climate is rather cool and the vegetation luxuriant, although the primeval forest has suffered much from clearings and casual wood-cutting. This progress of civilization also menaces the existence of the endemic species of deer (*Cervus kuhli*) which seems to have declined seriously in late years.

There is hardly any flat country in Bawean, the mountain spurs extending close to the sea. The island is inhabited by circa 42.000 people, half a dozen of which are Europeans. The principal village is Sangkapoera, on the S coast. Bawean is surrounded by an extensive and complicated system of coral reefs and shingle walls, rendering the access to the island very dangerous.

Literature: C. H. SIEGEL, Rapport over eene reis naar het eiland Bawean. Tijdschr. Binnenl. Bestuur, Vol. 20, 1901, p. 370–378.
J. E. JASPER, Het eiland Bawean en zijn bewoners. Tijdschr. Binnenl. Bestuur, Vol. 31, 1906, p. 231–280.
J. VAN ROON, Enkele aanteekeningen omtrent het eiland Bawean. Jaarversl. Topogr. Dienst (1916) Vol. 12, 1917, p. 264–273.
E. H. B. BRASCAMP, De boschgesteldheid van Bawean. Tectona, Vol. 16, 1923, p. 446–455.

31. *Madoera Island.* This largest of the satellite islands of Java is lying E of the N-coast of the main island, forming almost the direct continuation of it. Madoera is separated from Java by a narrow channel, the Trechter.

Its greatest length is 160 km, the greatest breadth 38 km. The entire surface measures 8118 geographical miles square.

It is a self governed Residency with nearly 2 million inhabitants, 1000 of which are Europeans.

With the exception of the shore region the island is hilly to even mountainous, more so by the sharp profile of the mountains than on account of their absolute altitude, the highest points, Goenoeng Pajoedan-daleman and G. Tamboekoe, attaining only 450 and 470 m respectively.

The island is chiefly composed of tertiary rock, soft marls and limestone alternating. The latter is arranged in 3 to 4 series of hills. In the lowlands quarternary deposits occur. Morphological and geological features point to the fact that Madoera is not only closely related to Java, especially to its eastern part, but even forms the immediate continuation of the physical conditions in the Javanese Residencies Japara-Rembang and Bodjonegoro, a continuation which cannot virtually be concealed by the interruption of the narrow passage between Madoera and the main island.

The Madoerese people are famous for their seafaring qualities, living largely on fishery and on coastal trade. For the rest cattle breeding is important whereas the Government salt production is a special industry of Madoera.

The very meagre primeval vegetation and the dry climate during the East monsoon thwart the molluscan fauna in this island, otherwise so favourable by its calcareous soil.

Literature: VAN DER PLAS, Boschtoestand op het eiland Madoera. Tectona, Vol. 8, 1915, p. 453–459.
J. S. BRANDTS BUYS, Madoera. Djawa, 1926, p. 369–374.

32. *Kangean Islands.* A few miles East of Madoera lies an archipelago of some 25 islands between 115°.10' and 115°.56' E and 6°.30' and 7°.18' S, belonging to the Residency of Madoera. Together the islands occupy nearly 700 km square. The main island, Kangean, stretches circa 50–60 km across in length and 10–20 km in breadth. In W-E direction the island is traversed by 2–3 undulating, anastomosing hill ranges, being the continuation of similar constructions in Madoera. The highest peaks are not exceeding 300–350 m altitude. Like in Madoera the hills are composed of marls and limestone, here and there forming caverns, some of which containing stalactite and stalagmite formations of a most exquisite beauty.

The central part of Kangean is covered by thick jungle. Towards the lower regions large allotments are planted with teakwood forests which are in charge of the Government. Along the coast there are vast mangrove bushes, alternating with drier regions where coconut culture is practised. These favourable wood conditions render the climate in Kangean rather mild, especially the East monsoon which is less hot and less dry here than in the neighbouring, deforested island of Madoera.

The population amounts to some 40.000 people, among which are less than a dozen Europeans. The natives are skilled seafarers and fishermen. Besides many are working in the forest service.

The Kangean islands are famous for the occurrence of a species of *Megapodius* (*M. reinwardtii*), being one of the most Western outposts of a bird family otherwise chiefly at home in Australia and New Guinea. For this reason the island of Saobi was proclaimed a nature reserve in 1926. The island of Sepandjang is inhabited by deer (*Cervus hippelaphus*) and monkeys (*Macaca irus*). In the caves bats are abundant.

Some of the islands are elevated coral reefs. Living reefs are nowadays traversing and surrounding the entire archipelago, rendering navigation in this region a most risky enterprise.

Literature: J. L. VAN GENNEP, Bijdrage tot de kennis van den Kangean-Archipel. Bijdr. Taal-, Land- en Volkenk. Ned. Ind. (6) Vol. 2, 1896, p. 89–108, with map.

A. REILINGH, De bosschen en het boschbedrijf in den Kangean-Archipel. Tectona, Vol. 12, 1919, p. 425–444.

P. A. BEYNON, Enkele aanteekeningen over den Kangean-Archipel. Weekbl. v. Indië, Vol. 16, 1919–1920, p. 51–53.

P. A. BEYNON, Ons wondere tropenland. Kangean in nieuwe banen geleid. Weekbl. v. Indië, Vol. 16, 1919–1920, p. 178–179.

C. O. VAN DER PLAS, De visscherij en de vischhandel in den Kangean- en Sapoedi-Archipel. Kol. Tijdschr. Vol. 9, 1920, p. 518–570 and 611–632.

————, Herinneringen aan Kangean. Indië, Geïll. Weekbl. Vol. 4 1920–1921, p. 725–729, 741–744, 762–766, 794–799, 811–814, Vol. 5, 1921–1922, p. 117–120, 153–157, 181–187, 202–205, 217–219.

F. J. APPELMAN, Iets over de „Gosong". Tropische Natuur, Vol. 27, 1938, p. 133–138.

33. *Noesa Baron.* On the South coast of Java, off the Bay of Poeger, close to the frontier between the residencies of Malang and Besoeki, at circa 113°.20′ E, lies the 6000 ha large island Noesa Baron, belonging to the Residency of Besoeki.

It consists entirely of limestone rock, difficult of access, rising to 313 m in the West part. The entire island is thickly clad with virgin forest and inhabited by a monkey (*Macaca irus*) and by a species of deer (*Cervus hippelaphus*) and some smaller mammals, but no large carnivorous animals.

Noesa Baron is not inhabited, but now and then natives visit the island in search of edible birds-nests. It is a nature reserve since 1920.

Literature: F. J. APPELMAN & G. F. H. W. RENGERS HORA SICCAMA, Noesa Baroeng, Natuurmonument tegenover Besoeki's Zuidkust. Drie Jaren Indisch Natuurleven, 11de Verslag (1936–1938) Ned. Indische Ver. Natuurbesch. 1939, p. 289–292.

34. *Noesa Kembangan*. Is a long, narrow island, close to the S-coast of Central Java and separated from the main island partly by a narrow channel and partly by a wider bay, Segara Anakan. It belongs to the Residency of Banjoemas.

The N-coast is low and swampy, fringed with tidal forest, the S-, E- and W-coasts are rocky and steep, with numerous cliffs and minute islands, among which the famous Karang Bolong where edible birds-nests are collected.

Although essentially of volcanic origin calcareous rock is projecting here and there. In this limestone region, close to the NW-shore, a dripstone cavern with magnificent stalactites and stalagmites is found.

The yearly amount of rain is high, circa 3800 mm on the average. Hence the vegetation is luxuriant. The W part of the island is a nature reserve since 1937.

Literature: A. H. BLAAUW, Noesa Kambangan, in: De Tropische Natuur in Schetsen en Kleuren. Amsterdam, 1913, p. 71–96.

35. *Trouwers Island*. Low island, covered by coconut trees and surrounded by a steep coral reef. There is no mangrove vegetation or other swampy land. The island is not inhabited. It belongs to the Residency of Bantam.

36. *Klapper Island*. Is swampy in the centre. The drier parts bear coconut trees and various shrubs. Like the preceding island it is not inhabited and it equally belongs to the Residency of Bantam.

III. SYSTEMATIC PART

Theodoxis angulosus (Récluz)

1843 RÉCLUZ, Proc. Zool. Soc. London, p. 173 (*Nerita*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 80 (*Neritina*).

Material examined:
  B. Museum Buitenzorg.
      Madoera Id., leg. P. A. OUWENS.

The species is not uncommon in the Eastern part of the Malay Archipelago and in the Philippines. As far as my information reaches it is not known from Java.

Theodoxis bicolor (Récluz)

1843 RÉCLUZ, Proc. Zool. Soc. London, p. 172 (*Nerita*).

1843 RÉCLUZ, Proc. Zool. Soc. London, p. 199 (*Nerita subpunctata*).

1879 MARTENS, in: MART.-CHEMN. N. Syst. Conch. Cab. Vol. 2, Part 10, p. 181–182, pl. 18, fig. 18 and 21 (*Neritina bicolor*), p. 179–181, pl. 18, fig. 19, 20, 22–24 (*Neritina subpunctata*).

Material examined:
  B. Museum Buitenzorg.
      Madoera Id., Pamakasan, leg. P. A. OUWENS.
Previous records in literature:
      Krakatau Id., 1890 BOETTGER. Ber. Senckenb. naturf. Ges. p. 163 (*Neritina subpunctata* var. *moluccensis*).
      Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 142 (*Neritina subpunctata* var. *moluccensis*).

The species is known from most of the islands of the Malay Archipelago including Java. It is also recorded from the Philippines. Madoera Id. is a new record.

Theodoxis corona (Linné)

1758 LINNÉ, Syst. Nat. Ed. X, p. 777 (*Nerita*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 79 (*Neritina brevispina*).

Material examined:
  B. Museum Buitenzorg.
      Madoera Id., Pamekasan, leg. P. A. OUWENS.
Previous records in literature:
      Noesa Kembangan, 1912 SCHEPMAN, Proc. malac. Soc. London, Vol. 10, p. 238 (*Neritina brevispina*).
      Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 264 (*Neritina brevispina*).

This species is one of the commonest Neritinids in the Malay Archipelago. It is also abundant in Java.

Theodoxis flavovirens (Von dem Busch)

1843 Vom dem Busch, in: Philippi, Abb. Conch. Vol. 1, Part 2, p. 26, pl. 1, fig. 23 (*Neritina*).
1897 Martens, in: Weber, Erg. Reise Nied. Ost Indien, Vol. 4, p. 81 (*Neritina*).

Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. Ouwens.

The specimens belong to the fa. *spinifera*. *Th. flavovirens* is not quite so common as the preceding species. It was known from Java since a considerable time.

Theodoxis oualaniensis (Lesson)

1831 Lesson, Voy. Coquille, Zool. Vol. 2, p. 379 (*Neritina*).
1897 Martens, in: Weber, Erg. Reise Nied. Ost Indien, Vol. 4, p. 82 (*Neritina ualanensis*).

Material examined:
    B. Museum Buitenzorg.
        Verlaten Id., Oct. 25, 1921, leg. K. W. Dammerman (var. *nigrobifasciata* Mrts).
        Madoera Id., Branta, leg. P. A. Ouwens.
    C. Museum Leiden.
        Madoera Id. leg. E. F. Jochim (var. *diremta* Mrts).
Previous records in literature:
        Verlaten Id., 1925 Van Benthem Jutting, Treubia, Vol. 6, p. 144 (*Neritina ualanensis* var. *nigrobifasciata*).
        Verlaten Id., 1929 Dammerman, Krakatau's New Fauna, p. 117 (*Neritina ualanensis* var. *nigrobifasciata*).

This little *Theodoxis* is one of the commonest species in the Malay Archipelago and beyond to the West and to the East. It is also known from Java.

Neritodryas cornea (Linné)

1758 Linné, Syst. Nat. Ed. X, p. 777 (*Nerita*).
1897 Martens, in: Weber, Erg. Reise Nied. Ost Indien, Vol. 4, p. 218 (*Neritina*).
1937 Riech, Arch. Naturgesch. N. F. Vol. 6, p. 76–77.

Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. Ouwens.

One of the commonest species in the Indo-Australian region. I have also records from Java.

Neritina (Dostia) violacea (Gmelin)

1790 GMELIN, Syst. Nat. Ed. XIII, p. 3685 (Nerita).
1822 LAMARCK, Anim. s. Vert. Vol. 6, Part 2, p. 186 (crepidularia)
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 218 (crepidularia).

Material examined:
  A. Museum Amsterdam.
      Edam Id., 1938, leg. J. D. F. HARDENBERG.
      Purmerend Id., 1938, leg. J. D. F. HARDENBERG.
      Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
  B. Museum Buitenzorg.
      Madoera Id., Pamekasan, leg. P. A. OUWENS.
      Madoera Id., Dec. 8, 1914, leg. P. A. OUWENS.

Neritina violacea is a species of the Western part of the Malay Archipelago: the three Greater Sunda Islands and a few of the Lesser Sunda Islands, but not in the Moluccas. Since long it has been stated in Java.

Neritina (Vittina) turrita (Gmelin)

1790 GMELIN, Syst. Nat. Ed. XIII, p. 3686 (Nerita).
1822 LAMARCK, Anim. s. Vert. Vol. 6, Part. 2, p. 185 (zigzag).
1834 QUOY & GAIMARD, Voy. Astrolabe, Zool. Vol. 3, p. 195 (Nerita communis).
1937 RIECH, Arch. Naturgesch. N. F. Vol. 6, p. 80.

Material examined:
  A. Museum Amsterdam.
      Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
  B. Museum Buitenzorg.
      Madoera Id., Pamekasan, leg. P. A. OUWENS.

Previous records in literature:
      Noesa Kembangan, 1879 MARTENS, in: MART.–CHEMN. N. Syst. Conch. Cab. Vol. 2, Part 10, p. 105 (semiconica).
      Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 264.

One of the commonest species in the Indo-Australian region, and a settler in Java since a very long time.

Neritina (Vittina) variegata Lesson

1831 LESSON, Voy. Coquille, Zool. Vol. 2, p. 378.

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 78.

1937 RIECH, Arch. Naturgesch. N. F. Vol. 6, p. 77–78.

Material examined:
    A. Museum Amsterdam.
        Noesa Kembangan, June 1931, leg. Mrs. A. C. VAN HEURN.
        Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
    C. Museum Leiden.
        Madoera Id., Ketapang, leg. E. F. JOCHIM.

A very common species, from the Nicobar Ids. in the West to New Caledonia and Fiji in the East, including Java.

Neritina (*Neritina*) *pulligera* (Linné)

1767 LINNÉ, Syst. Nat. Ed. XII, p. 1253 (*Nerita*).

1825 SOWERBY, Cat. Shells Tankerville, App. p. XI (*canalis*).

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 81 (*iris*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 77 (*pulligera* and *iris*).

1937 RIECH, Arch. Naturgesch. N. F. Vol. 6, p. 73–74.

Material examined:
    A. Museum Amsterdam.
        Noesa Kembangan, freshwater near Djoembling, Febr. 18, 1929, leg. DENIN.
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.
        Madoera Id., mouth of Madjoengan and Mangoenan rivers, leg. P. A. OUWENS.
Previous records in literature:
        Duizend Ids., 1891 BOETTGER, Ber. Senck. naturf. Ges. p. 248 (*iris*).
        Duizend Ids., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 263 (*iris*).

Neritina pulligera is very abundant in the entire Indo-Malay region, including Java.

Neritina (*Neritina*) *squamipicta* (Récluz)

1843 RÉCLUZ, Proc. Zool. Soc. London, p. 169 (*Nerita*).

1879 MARTENS, in: MART.-CHEMN. N. Syst. Conch. Cab. Vol. 2, Part 10, p. 62–63, pl. 5, fig. 9–11 (*Neritina*).

Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamakasan, leg. P. A. OUWENS.

The species is not a common one. It is only recorded from the Philippines and from Java. Madoera Id. is a new record.

Septaria cumingiana (Récluz)

1843 RÉCLUZ, Proc. Zool. Soc. London, p. 157 (*Navicella*).
1937 RIECH, Arch. Naturgesch. N. F. Vol. 6, p. 67–68.

Material examined:
  B. Museum Buitenzorg.
        Madoera Id., Tjerek, leg. P. A. OUWENS.

Septaria cumingiana is widely distributed in the Indo-Australian region and is also known from Java.

Septaria suborbicularis (Sowerby)

1825 SOWERBY, Cat. Shells, Tankerville, App. p. X (*Navicella*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 84.

Material examined:
  A. Museum Amsterdam.
        Amsterdam Id., 1938, leg. J. D. F. HARDENBERG.
        Middelburg Id., 1938, leg. J. D. F. HARDENBERG.
        Noesa Kembangan, March 1911, leg. E. JACOBSON.
  B. Museum Buitenzorg.
        Madoera Id., Tjerek, leg. P. A. OUWENS.
        Noesa Kembangan, Febr. 1929, leg. DENIN.
  C. Museum Leiden.
        Noesa Kembangan, March 1911, leg. E. JACOBSON.
Previous records in literature:
        Noesa Kembangan, 1912 SCHEPMAN, Proc. malac. Soc. London, Vol. 10, p. 238.
        Noesa Kembangan, 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 39.

It is one of the commonest freshwater gastropods in the Indo-Malay region, including Java.

Septaria tessellata (Lamarck)

1822 LAMARCK, Anim. s. Vert. Vol. 6, Part 2, p. 182 (*Navicella*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 86.
1937 RIECH, Arch. Naturgesch. N. F. Vol. 6, p. 68–69.

Material examined:
  B. Museum Buitenzorg.
        Madoera Id., Tjerek, leg. P. A. OUWENS.

Occurs frequently in the Indo-Australian region between India and the Fiji Ids., and is also recorded from Java. Madoera is a new locality.

Hydrocena (Georissa) javana (Möllendorff)

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 97 (*Georissa*).

Material examined:
   A. Museum Amsterdam.
         Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.

The species is known from Sumatra, Java and Bali. Intensified research of the moss-fauna will certainly reveal it from more islands.

Geophorus rollei (Sykes)

1901 SYKES, Journ. Malac. Vol. 8, p. 59–60, with fig. (*Helicina*).

Material examined:
   A. Museum Amsterdam.
         Kangean Ids., leg. ROLLE.
Previous records in literature:
         Kangean Ids., 1901 SYKES, l.c.

Only known from the Kangean Islands.

Leptopoma altum Möllendorff

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 90.
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 17–18.

Material examined:
   C. Museum Leiden.
         Noesa Kembangan, March 1911, leg. E. JACOBSON.
Previous records in literature:
         Noesa Kembangan, 1912 SCHEPMAN, Proc. malac. Soc. London. Vol. 10, p. 237.
         Noesa Kembangan, 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11, p. 81.

Leptopoma altum is known from Java and Noesa Kembangan.

Leptopoma vitreum (Lesson)

1830 LESSON, Voy. Coquille, Zool. Vol. 2, p. 346, pl. 13, fig. 6 (*Cyclostoma*).
1867 MARTENS, Ostas. Landschn. p. 143–147.
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 15.

Material examined:
   A. Museum Amsterdam.
         Sebesi Id., April 25, 1921, leg. K. W. DAMMERMAN.
         Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16 1927, leg. P. F. FRANCK.
         Noesa Kembangan, Febr. 1931, leg. K. B. BOEDIJN (note of collector: light-green during life).
         Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
         Trouwers Id., Febr. 18, 1932, leg. DARNA and SAÄN.

Klapper Id., Febr. 18–23, 1932, leg. DARNA and SAÄN.
Meeuwen Id., March 30, 1932, leg. P. F. FRANCK.
B.   Museum Buitenzorg.
Sebesi Id., April 25, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143.
Noesa Kembangan, 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67, p. 174.

Very common in several islands of the Malay Archipelago, especially in the Moluccas. It is also known from Java.

Japonia trochulus (Martens)

1867 MARTENS, Ostas. Landschn. p. 141–142 (*Cyclophorus*).
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 57–58.

Material examined:
A.   Museum Amsterdam.
Bawean Id., Southern part, soil fauna, May 1928, leg. K. W. DAMMERMAN.
Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1927, leg. P. F. FRANCK.
Noesa Kembangan, June 1931, leg. Mrs. A. C. VAN HEURN.

The species is known from Java since 1859 when ZOLLINGER mentioned it (Natuurk. Tijdschr. Ned. Indië, Vol. 18, p. 424) as *Cyclostoma trochulus* Mousson (nomen nudum).

Cyclophorus perdix (Broderip & Sowerby)

1830 BRODERIP & SOWERBY, Zool. Journ. Vol. 5, p. 50 (*Cyclostoma*).
1867 MARTENS, Ostas. Landschn. p. 136–138.
1902 KOBELT, Cyclophcridae in: Tierreich, Vol. 16, p. 131.

Material examined:
A.   Museum Amsterdam.
Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1927, leg. P. F. FRANCK.
Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
B.   Museum Buitenzorg.
Madoera Id., Pegantenan, leg. P. A. OUWENS.
Noesa Kembangan, Nature Reserve Gligir, Febr. 1927, leg. P. F. FRANCK.
C.   Museum Leiden.
Noesa Kembangan, near waterfall, June 1931, leg. W. C VAN HEURN.
Previous records in literature.
Krakatau Id., 1867 MARTENS, l.c.

Krakatau Id., 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 61
(*perdix* and *zollingeri*).
Krakatau Id., 1931 RENSCH, Zool. Jahrb. (Syst.) Vol. 61, p. 371.
Krakatau Id., 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 741.
Noesa Kembangan, 1935, PARAVICINI, Arch. Moll. Kunde, Vol.
67, p. 174.

The references to Krakatau are based on material found before
the eruption of 1883. After this catastrophe *Cyclophorus perdix* is
no longer found in Krakatau. The species is very abundant in
Java and Bali.

Cyclophorus rafflesi (Broderip & Sowerby)

1830 BRODERIP & SOWERBY, Zool. Journ. Vol. 5, p. 50 (*Cyclostoma*).
1867 MARTENS, Ostas. Landschn. p. 132–133.
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 117–118.

Material examined:
B. Museum Buitenzorg.
Madoera Id., Pegantenan, leg. P. A. OUWENS.
C. Museum Leiden.
Noesa Kembangan, near swimming pool, June 1931, leg. W. C.
VAN HEURN.
Previous records in literature:
Noesa Kembangan, 1867 MARTENS, l.c.
Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg.
Vol. 31, p. 243.

The species is frequent in Java, especially in the West and
Central part.

Cyclotus (Opisthoporus) corniculum (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 267
(*Cyclostoma*).
1849 MOUSSON, Land-und Süssw. Moll. Java, p. 51–52, pl. 5,
fig. 11 (*Cyclostoma*).
1867 MARTENS, Ostas. Landschn. p. 112 (*Opisthoporus*).
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 214–215.

Material examined:
B. Museum Buitenzorg.
Noesa Kembangan, Nature Reserve Gligir, Febr. 15, 1927, leg.
P. F. FRANCK.
Previous records in literature:
Noesa Kembangan, 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67,
p. 174.

The species is a common ground-dweller in the jungle of Java.

Cyclotus kangeanus Schepman

1909 SCHEPMAN, Siboga Exp. Monogr. 49–1–b, p. 198, pl. 12, fig. 10.

Material examined:
A. Museum Amsterdam.
Kangean. Ids., leg. FRUHSTORFER.
Kangean Ids., leg. Siboga Expedition.
Previous records in literature:
Kangean Ids., 1909 SCHEPMAN, l.c.

The species is only known from the Kangean Islands.

Pupina superba Pfeiffer

1855 PFEIFFER, Proc. Zool. Soc. London, p. 118.
1867 MARTENS, Ostas. Landschn. p. 156.
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 322–323.

Material examined:
A. Museum Amsterdam.
Sebesi Id., 700 m alt., April 25, 1921, leg. K. W. DAMMERMAN.
B. Museum Buitenzorg.
Sebesi Id., April 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 144.

The species is only recorded from Sumatra and Sebesi.

Pupina treubi Boettger

1890 BOETTGER, Ber. Senckenb. naturf. Ges. p. 157, pl. 6, fig. 8.
1902 KOBELT, Cyclophoridae in: Tierreich, Vol. 16, p. 324.

Material examined:
B. Museum Buitenzorg.
Madoera Id., Djoemiang, leg. P. A. OUWENS.

The species is known from Java since 1890. Recently I could identify a specimen from Enggano Island, which was not included in my paper on the Non-marine Mollusca from Enggano Island (Treubia, Vol. 16, 1937, p. 47–50). Madoera is a new record.

In 1909 E. JACOBSON mentioned a *Pupina* (or *Porocallia*?) sp., found by him in Krakatau Id. (Jaarversl. Topogr. Dienst Ned. Indie, Jaarg. 4, 1908, (1909) list opposite p. 206) and which was quoted by me (Treubia, Vol. 6, 1925, p. 144).
The shell was presented to the Basle Natural History Museum. At my

request the late Dr. J. Roux, Curator of the Museum, looked for it in the collections under his charge and wrote to me d.d. June 29, 1925: (translated by me, W. S. S. v. B. J.) "The so-called *Pupina* was a *Pupa*, undeterminable, which was badly preserved and was thrown away".

I think it advisable to leave out the reference for good, as it gives no information at all, on the contrary, causes further confusion.

Viviparus javanicus (Von dem Busch)

1844 Von dem Busch, in: Philippi, Abb. Conch. Vol. 1, Part 5, p. 114 (*Paludina*).

1897 Martens, in: Weber, Erg. Reise Nied. Ost Indien, Vol. 4, p. 21–24 (*Vivipara*).

Material examined:
    A. Museum Amsterdam.
        Bawean Id., Sangkapoera, Aug. 11, 1920, leg. H. C. Delsman.
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. Ouwens.
Previous records in literature:
        Madoera Id., 1897 Martens, l.c.
        Madoera Id., 1922 Van Heurn & Paravicini, Natuurk. Tijdschr. Ned. Indië, Vol. 82, p. 31.
        Madoera Id., 1923 Oostingh, Meded. Landb. Hoogesch. p. 55.

One of the commonest freshwater shells in the Greater Sunda Islands and several of the Lesser Sunda Islands. I have no records from the Moluccas. Since many years it is also known from Java.

Pila conica (Gray)

1828 Gray, in: Wood, Index Test. Suppl. p. 29, pl. 7, fig. 22 (*Ampullaria*).

1849 Mousson, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 268 (*Ampullaria scutata*).

1849 Mousson, Land- & Süssw. Moll. Java, p. 60, pl. 8, fig. 2 (*Ampullaria scutata*).

1897 Martens, in: Weber, Erg. Reise Nied. Ost Indien, Vol. 4, p. 18–19 (*Ampullaria scutata*).

Material examined:
    A. Museum Amsterdam.
        Hoorn Id., 1938, leg. J. D. F. Hardenberg.
        Enkhuizen Id., 1938, leg. J. D. F. Hardenberg.
        Kerkhof Id., 1938, leg. J. D. F. Hardenberg.
        Purmerend Id., 1938, leg. J. D. F. Hardenberg.
        Edam Id., 1938, leg. J. D. F. Hardenberg.
        Karimon Djawa Ids., from sawahs, freshwater, May 1926, leg. K. W. Dammerman.

Karimon Djawa Ids., from rivulet near kampong, freshwater, May 1926, leg. K. W. DAMMERMAN.

Karimon Djawa Ids., from sawah, Nov. 30, 1930, leg. M. A. LIEFTINCK.

Bawean Id., Sangkapoera, Aug. 11, 1920, leg. H. C. DELSMAN.

Bawean Id., Southern part, from rivulet near the shore, May 1928, leg. K. W. DAMMERMAN.

Noesa Kembangan, leg. L. DE PRIESTER.

C. Museum Leiden.

Madoera Id., leg. Mrs. MANGOLD.

The species is abundant in the three Greater Sunda Islands and in several of the Lesser Sunda Islands. I have no records from the Moluccas. Since many years it is known from Java. All the above mentioned islands are new records.

Pila polita (Deshayes)

1830 DESHAYES, Encycl. Méth. (Vers) Vol. 2, Part 1, p. 31 (*Ampullaria*).

Material examined:

A. Museum Amsterdam.

Noesa Kembangan, leg. L. DE PRIESTER.

The species is not uncommon in Central and East Java. I have no records beyond this island. Noesa Kembangan is a new locality.

Stenothyra ventricosa (Quoy & Gaimard)

1834 QUOY & GAIMARD, Voy. Astrolabe, Zool. Vol. 3, p. 173, pl. 58, fig. 6–8 (*Paludina*).

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 63, pl. 8, fig. 6 (*Paludestrina*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 210–211, pl. 9, fig. 7 (*moussoni*).

1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 399–400.

Material examined:

A. Museum Amsterdam.

Verlaten Id., Dec. 1933, leg. K. W. DAMMERMAN.

B. Museum Buitenzorg.

Verlaten Id., Dec. 1933, leg. K. W. DAMMERMAN.

Previous records in literature:

Verlaten Id., 1937 VAN BENTHEM JUTTING, Zool. Meded. Leiden, Vol. 20, p. 104.

The species is recorded besides from Java, Soembawa, Soemba and Celebes.

Bithynia truncata (Eyd. & Soul.)

1852 EYDOUX & SOULEYET, Voy. Bonite, Zool. Vol. 2, p. 548,
   pl. 31, fig. 22–24 (*Paludina*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 25–26, pl. 9, fig. 11, 11–b.
1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 398–399.

Previous records in literature:
   Madoera Id., 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67, p. 175.

The species is known from India, Malaya, Sumatra, Java,
Madoera, Lombok, Soembawa, Soemba and Celebes.

Assiminea borneensis (Issel)

1874 ISSEL, Ann. Mus. Civ. St. Nat. Genova, Vol. 6, p. 451–452,
   pl. 7, fig. 13–15 (not 16–18) (*Amnicola*).

Material examined:
   A. Museum Amsterdam.
      Leiden Id., 1937, leg. J. D. F. HARDENBERG.

Being known from Borneo since ISSEL's original diagnosis the
species has now been recorded also from coral shingle from the
Island of Leiden.

Assiminea brevicula (Pfeiffer)

1854 PFEIFFER, Proc. Zool. Soc. London, p. 306 (*Hydrocena*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 213–214.

Material examined:
   A. Museum Amsterdam.
      Kerkhof Id., 1938, leg. J. D. F. HARDENBERG.

Assiminea brevicula var. *miniata* (Martens)

1866 MARTENS, Ann. Mag. Nat. Hist. (3) Vol. 17, p. 204
   (*Assiminea miniata*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 214, pl. 9, fig. 21, pl. 10, fig. 3.

Material examined:
   A. Museum Amsterdam.
      Amsterdam Id., 1938, leg. J. D. F. HARDENBERG.

Both the principal form and its red variety are known from
the brackish coasts along a great many islands of the Malay
Archipelago, including Java. Furthermore from Malaya, Siam,
India, China and the Philippines.

Assiminea sinensis Nevill

1880 Nevill, Journ. As. Soc. Bengal, Vol. 49, Part. 2, p. 161.
1934 Van Benthem Jutting, Misc. Zool. Sum. No. LXXXIV–LXXXV, p. 7–8, fig. 5–7.

Material examined:
  A. Museum Buitenzorg.
        Noesa Kembangan, 1929, leg. W. G. N. van der Sleen.

It is the second record from the Malay Archipelago (Van Benthem Jutting, l.c.) So far it is not known from Java.

Assiminea subeffusa Boettger

1887 Boettger, Jahrb. d. Malak. Ges. Vol. 14, p. 205, pl. 6, fig. 11.

Material examined:
  A. Museum Amsterdam.
        Noesa Kembangan, 1929, leg. W. G. N. van der Sleen.

The shells agree satisfactorily with the diagnosis and the – rather poor – figure of Boettger's paper. Therefore I do not hesitate to bring them to *Assiminea subeffusa*, although I had no other material to compare with. The species was originally described from Hongkong. The above-mentioned specimens are the first from the Malay Archipelago.

Omphalotropis (Stenotropis) columellaris Quadras & Möllendorff
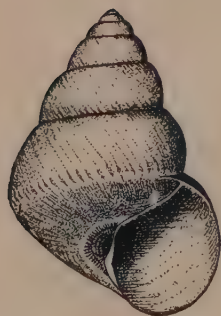1893 Quadras & Möllendorff, Nachr. Blatt, Vol. 25, p. 183.

Material examined:
  A. Museum Amsterdam.
        Noordwachter Id., Sept. 8, 1921, leg. K. W. Dammerman.
        Klein Kombuis Id., Nov. 11, 1920, leg. K. W. Dammerman.

The species was only known from the Philippines so far. The Malay Archipelago is a new record. As it was never figured I take this opportunity to give a drawing of a shell from Noordwachter (fig. 1). Dimensions of 20 specimens from the same set in 1/10 mm:

Fig. 1. *Omphalotropis columellaris* Quadras & Möllendorff. x 10. Noordwachter Id., Sept. 8, 1921.

Height:	40 39 39 38 38 36 35 35 35 34 34 33 33 33 33 32 32 32 32 31
Breadth:	29 28 28 26 26 26 26 25 25 26 25 26 25 24 24 24 24 24 23 23
No. of whorls:	6¾ 6¾ 6¾ 6½ 6¼ 6¼ 6¼ 6¼ 5¾ 6 5¾ 5½ 6 5¾ 5½ 6 5¾ 5¾ 5¾ 5¾

Brotia testudinaria (Von dem Busch)

1842 VON DEM BUSCH, in: PHILIPPI, Abb. Conch. Vol. 1, p. 3, pl. 1, fig. 14 (*Melania*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 31 (*Melania*).

Material examined:
    A. Museum Amsterdam.
       Bawean Id., Telaga Kastobo, June 2, 1920, leg. H. C. DELSMAN.
       Bawean Id., Sangkapoera, Aug. 11, 1920, leg. H. C. DELSMAN.
       Bawean Id., Telaga Kastobo, 250 m alt., Nov. 25–27, 1927, leg. J. H. COERT (note of collector: very abundant on stones, on wood, on the bottom of the lake, in the latter case, however, invariably dead; nearly always the shell is more or less corroded).
       Noesa Kembangan, March 1922, leg. W. M. DOCTERS VAN LEEUWEN.
    B. Museum Buitenzorg.
       Madoera Id., Pamekasan, leg. P. A. OUWENS.
       Bawean Id., Southern part, from streamlet near the beach, May 1928, leg. K. W. DAMMERMAN.
Previous records in literature:
       Madoera Id., 1934 RENSCH, Trop. Binnengew. Vol. 5, p. 24.

The species was only known from South Sumatra, Java and Madoera. The islands of Bawean and Noesa Kembangan are new records.

Thiara (*Plotia*) *scabra* (Müller)

1774 MÜLLER, Hist. Verm. Vol. 2, p. 136 (*Buccinum*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 62–65, pl. 4, fig. 6–12, pl. 9, fig. 8–9 (*Melania*).

Material examined:
    A. Museum Amsterdam.
       Amsterdam Id., 1938, leg. J. D. F. HARDENBERG.
       Bawean Id., Southern part, from rivulet near the shore, May 1928, leg. K. W. DAMMERMAN.
    B. Museum Buitenzorg.
       Madoera Id., Pamekasan, leg. P. A. OUWENS (var. *mutica* Marts).

One of the commonest species in the entire Indo-Australian region, including Java.

Thiara (Tarebia) granifera (Lamarck)

1816 LAMARCK, Encycl. Méth. (Vers) pl. 458, list p. 12 (*Melania*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 71–72 (*Melania*).
1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 404-408 (*Melania*).

Material examined:
    A. Museum Amsterdam.
        Bawean Id., Telaga Kastobo, June 2, 1920, leg. H. C. DELSMAN.
        Bawean Id., Southern part, from rivulet near the shore, May 1928, leg. K. W. DAMMERMAN.
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

One of the commonest species in India, the Malay Archipelago till New Guinea, including Java.

Thiara (Stenomelania) crenulata (Deshayes)

1838 DESHAYES, in: LAMARCK, Anim. s. Vert. Ed. II, Vol. 8, p. 434 (*Melania*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 45–46 (*Melania*).

Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

The species is widely distributed throughout the Indo-Malay region from India, via the Malay Archipelago and the Philippines, to the Solomon Islands. It is also known from Java. Madoera Id. is a new record.

Thiara (Stenomelania) obesula (Brot)

1874 BROT, Melaniaceen, in: MART.-CHEMN. N. Syst. Conch. Cab. Vol. 1, Part 24, p. 121, pl. 15, fig. 8 (*Melania*).

Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

The species was originally described from Java. I have not seen other records since that time. Madoera is a new locality.

Thiara (Stenomelania) plicaria (Born)

1780 BORN, Test. Mus. Caes. Vindob. p. 389, pl. 16, fig. 14 (*Helix*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
    p. 41–42 (*Melania*).
1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 413–414 (*Melania*).

Material examined:
    B. Museum Buitenzorg:
        Madoera Id., Pamekasan, leg. P. A. OUWENS.
    C. Museum Leiden.
        Madoera Id., leg. E. F. JOCHIM (s. n. *hastula* and *terebriformis*).
Previous records in literature:
        Madoera Id., 1934 RENSCH, l.c.

Thiara plicaria is a common species in several islands of the
Malay Archipelago between Sumatra and New Pommerania,
including Java and Madoera.

Thiara (*Stenomelania*) *punctata* (Lamarck)

1822 LAMARCK, Anim. s. Vert. Vol. 6, Part 2, p. 165 (*Melania*).
1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 411–413 (*Melania*).
Material examined:
    B. Museum Buitenzorg.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.
    C. Museum Leiden.
        Madoera Id., leg. E. F. JOCHIM.

The species is widely distributed throughout the Indo-Malay
region from India in the West till New Guinea in the East,
including Java.

Thiara (*Melanoides*) *tuberculata* (Müller)

1774 MÜLLER, Hist. Verm. Vol. 2, p. 191 (*Nerita*).
1849 MOUSSON, Land- & Süssw. Moll. Java, p. 70–71, pl. 11,
    fig. 8 (*Melania unifasciata*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
    p. 56–59 (*Melania*).
1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 400–404 (*Melania*).
Material examined:
    A. Museum Amsterdam.
        Amsterdam Id., 1938, leg. J. D. F. HARDENBERG.
        Karimon Djawa Ids., from rivulet near kampong, freshwater,
            May 1926, leg. K. W. DAMMERMAN.
        Karimon Djawa Ids., Poeloe Mendjangan Besar, on Sonneratia,
            Nov. 22, 1930, leg. M. A. LIEFTINCK.
        Bawean Id., Telaga Kastobo, June 2, 1920, leg. H. C. DELSMAN.
        Bawean Id., Southern part, from rivulet near the shore, May
            1928, leg. K. W. DAMMERMAN.

Bawean Id., Telaga Kastobo, 250 m alt., Nov. 25–27, 1937, leg. J. H. COERT.

B. Museum Buitenzorg.

Sebesi Id., Northern part, April 1921, leg. H. BOSCHMA.

Sebesi Id., Northern part, April 1921, leg. K. W. DAMMERMAN (var. *seminuda* Mrts).

Sebesi Id., Northern part, from brackish water swamp, April 1921, leg. H. BOSCHMA (var. *seminuda* Marts).

Verlaten Id., in brackish water lake, Jan. 1933, leg. K. W. DAMMERMAN.

Previous records in literature:

Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 144 (*Mel. tuberculata* and *Mel. tuberculata* var. *seminuda*).

Madoera Id., 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67, p. 175 (*Melania unifasciata*).

The species with its numerous varieties is distributed in Africa, India, the Malay Archipelago to North Australia and several South Sea islands. It is since long an inhabitant of Java. Amsterdam Id., the Karimon Djawa group and Bawean Id. are new records.

Laemodonta imperforata (Adams)

1853 ADAMS, Proc. Zool. Soc. London, p. 120–121 (*Plecotrema*).
1856 PFEIFFER, Monogr. Auric. Viv. p. 106–107 (*Plecotrema*).

Previous records in literature:

Madoera Id., 1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 149 (*Plecotrema*).

Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 248 (*Plecotrema*).

Only known from the Philippines and Madoera.

Allochroa conica (Pease)

1862 PEASE, Proc. Zool. Soc. London, p. 242 (*Laimodonta*).
1876 PFEIFFER, Monogr. Pneumop. Suppl. 3, p. 319–320 (*Melampus*).

Material examined:

B. Museum Buitenzorg.

Kerkhof Id., April 29, 1928, leg. J. VERWEY.

The species is known from the Philippines, New Caledonia, Polynesia, New Guinea and the Aroe Islands (TAPPARONE CANEFRI, Ann. Mus. Civ. St. Nat. Genova, Vol. 19, 1883, p. 240). I have no records from Java. Kerkhof Id. is a new locality.

Auriculastra subula (Quoy & Gaimard) ·

1832 QUOY & GAIMARD, Voy. Astrolabe, Zool. Vol. 2, p. 171,
  pl. 13, fig. 39, 40 (*Auricula*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
  p. 158–160.

Material examined:
  A. Museum Amsterdam.
    Noesa Kembangan, 1929, leg. W. G. N. VAN DER SLEEN.

Auriculastra subula is recorded from Bengal to New Caledonia
and the Sandwich Ids, and occurs in most of the islands between
where some collecting has been done. However, we have no
records from Java. Noesa Kembangan is a new locality.

Melampus fasciatus (Deshayes)

1830 DESHAYES, Encycl. Méth. (Vers) Vol. 2, p. 90 (*Auricula*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
  p. 161–163, pl. 8, fig. 4 (*Auricula*).

Material examined:
  A. Museum Amsterdam.
    Alkmaar Id., 1938, leg. J. D. F. HARDENBERG.
    Kerkhof Id., 1938, leg. J. D. F. HARDENBERG.
    Purmerend Id., 1938, leg. J. D. F. HARDENBERG.
    Middelburg Id., 1938, leg. J. D. F. HARDENBERG.
    Rotterdam Id., 1938, leg. J. D. F. HARDENBERG.
    Karimon Djawa Ids., Poeloe Kemoedjan, under stones and
      leaves in coconut garden close to the shore, Nov. 30, 1930,
      leg. M. A. LIEFTINCK.
    Noesa Kembangan, 1929, leg. W. G. N. VAN DER SLEEN.
  B. Museum Buitenzorg.
    Madoera Id., Tjerek, leg. P. A. OUWENS.
  C. Museum Leiden.
    Madoera Id., leg. Mrs. MANGOLD.
Previous records in literature:
    Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 63, p. 222.

A very common species in the entire Malay Archipelago and
beyond it to Bengal in the West and Fiji in the East. It is also
known from Java.

Melampus luteus (Quoy & Gaimard)

1832 QUOY & GAIMARD, Voy. Astrolabe, Zool. Vol. 2, p. 163,
  pl. 13, fig. 25–27 (*Auricula*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
  p. 163–164.

Material examined:
- B. Museum Buitenzorg.
  - Madoera Id., Tjerek, leg. P. A. OUWENS.
- C. Museum Leiden.
  - Madoera Id., leg. Mrs. MANGOLD.

Previous records in literature:
- Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 63, p. 222.
- Noesa Baron, 1849 MOUSSON, Land- & Süssw. Moll. Java, p. 48 (*Auricula*).
- Noesa Baron, 1897 MARTENS, l.c.
- Noesa Baron, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 249.

A species with a wide distribution in the Indo-Australian region, including Java, about as abundant as *M. fasciatus*. Madoera is a new record.

Melampus variabilis Gassies

1863 GASSIES, Faune Conch. Nouv. Caléd. p. 65, pl. 6, fig. 8.

Material examined:
- A. Museum Amsterdam.
  - Karimon Djawa Ids., Poeloe Kemoedjan, under leaves and stones in coconut garden close to the shore, Nov. 30, 1930, leg. M. A. LIEFTINCK.

The species was not known before in our East Indian Archipelago. The specimen from Karimon Djawa Ids. agrees so well with the original description and with some shells from New Caledonia in our Museum, that I do not hesitate to bring it under this name.

Cassidula auris-felis (Bruguière)

1789 BRUGUIÈRE, Encycl. Méth. (Vers) Vol. 1, p. 343, pl. 460, fig. 5a–b (*Bulimus*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 141–142, pl. 8, fig. 12–14.

Material examined:
- A. Museum Amsterdam.
  - Kerkhof Id., 1938, leg. J. D. F. HARDENBERG.
  - Puimerend Id., 1938, leg. J. D. F. HARDENBERG.
- B. Museum Buitenzorg.
  - Madoera Id., Djoemiang and Tjerek, leg. P. A. OUWENS.
  - Madoera Id., Tjerek, leg. P. A. OUWENS.
- C. Museum Leiden.
  - Madoera Id., leg. E. F. JOCHIM.

Previous records in literature:
>Pandjang Id., near Japara, 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 143–144.

A very common species in the Western part of the Malay Archipelago, including Java. According to VON MARTENS (l.c.) it is missing in New Guinea and Polynesia, but our Museum possesses a recent sample from Kaimana, West New Guinea.

Cassidula labio Möllendorff

1887 MÖLLENDORFF, Jahrb. d. Malak. Ges. Vol. 14, p. 282–283, pl. 8, fig. 14–14b.

Material examined:
  A. Museum Amsterdam.
>Kerkhof Id., April 29, 1928, leg. J. VERWEY.
  B. Museum Buitenzorg.
>Kerkhof Id., April 29, 1928, leg. J. VERWEY.

The species was only known before from the Philippines and Java.

Cassidula mustelina (Deshayes)

1830 DESHAYES, Encycl. Méth. (Vers) Vol. 2, p. 92 (*Auricula*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 144–145, pl. 8, fig. 5.

Material examined:
  A. Museum Amsterdam.
>Purmerend Id., 1938, leg. J. D. F. HARDENBERG.
  B. Museum Buitenzorg.
>Madoera Id., Djoemiang, leg. P. A. OUWENS.
>Madoera Id., Tjerek, leg. P. A. OUWENS.
Previous records in literature:
>Noesa Kembangan, 1897 MARTENS (l.c.).
>Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 248.

A very common species in the entire Malay Archipelago and adjacent regions. It is also known from Java since a long time. Madoera is a new record.

Pythia castanea (Lesson)

1831 LESSON, Voy. Coquille, Zool. Vol. 2, p. 336, pl. 10, fig. 7 (*Scarabus*).
1883 TAPPARONE CANEFRI, Ann. Mus. Civ. St. Nat. Genova, Vol. 19, p. 235.

Material examined:
  B. Museum Buitenzorg.
        Madoera Id., Tjerek, leg. P. A. Ouwens.

The species is known from Waigeoe Id. and the Philippines. So far I have not seen records from Java. Madoera is a new locality.

Pythia chrysostoma Tapparone Canefri

1883 TAPPARONE CANEFRI, Ann. Mus. Civ. St. Nat. Genova, Vol. 19, p. 237–238, pl. 1, fig. 25–27.

Material examined:
  A. Museum Amsterdam.
        Krakatau Id., Dec. 1919, leg. K. W. DAMMERMAN.
        Krakatau Id., April 1920, leg. K. W. DAMMERMAN.
  B. Museum Buitenzorg.
        Krakatau Id., Dec. 1919, leg. K. W. DAMMERMAN.
        Krakatau Id., South-Eastern part, Oct. 1933, leg. K. W. DAM-
          MERMAN.
        Verlaten Id., Dec. 1919, leg. K. W. DAMMERMAN.

The species was originally described from the South-coast of New Guinea. In later years several stations more to the West increased its area of distribution, but so far I have no records from Java.

In my report on Molluscs from the Krakatau Islands (Treubia, Vol. 6, 1925, p. 140–145) I erroneously brought the 1919-samples under the name *Pythia scarabaeus* (L.). This will be discussed further under that species.

Pythia pantherina (Adams)

1850 ADAMS, Proc. Zool. Soc. London, p. 152 (*Scarabus*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 136–138.

Material examined:
  A. Museum Amsterdam.
        Prinsen Id., soil fauna, Jan. 1922, leg. K. W. DAMMERMAN (juve-
          nile shells, probably this species).
        Sebesi Id., North part, April 1921, leg. K. W. DAMMERMAN.
        Verlaten Id., Dec. 1919, leg. K. W. DAMMERMAN.
        Verlaten Id., North part, Sept. 27, 1920, leg. K. W. DAMMERMAN.
        Verlaten Id., North part, Aug. 1930, leg. K. W. DAMMERMAN.
        Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
        Noordwachter Id., Sept. 1921, leg. H. BOSCHMA.

Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.

Karimon Djawa Ids., Poeloe Kemoedjan, May 16, 1926, leg.
  P. F. FRANCK.

Noesa Kembangan, leg. L. DE PRIESTER.

Klapper Id., Febr. 18–23, 1932, leg. DARNA and SAÄN.

B.   Museum Buitenzorg.

Verlaten Id., Dec. 1919, leg. K. W. DAMMERMAN.

Previous records in literature:

Verlaten Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
  p. 144.

Verlaten Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 114.

Noesa Baron, 1849 MOUSSON, Land- & Süssw. Moll. Java, p. 49,
  pl. 5, fig. 10 (*Scarabus pyramidatus* var. *javanicus*).

Noesa Baron, 1860 ZOLLINGER, Natuurk. Tijdschr. Ned. Indië,
  Vol. 21, p. 317 (*Scarabus pyramidatus* var.).

Noesa Baron, 1874 ISSEL, Ann. Mus. Civ. St. Nat. Genova, Vol. 6,
  p. 424 (*Scarabus*).

Noesa Baron, 1897 MARTENS, l.c.

Noesa Baron, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg,
  Vol. 31, p. 247.

Noesa Baron, 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 143.

A very common species inhabiting most of the islands in the
Malay Archipelago, including Java.

Pythia plicata (Férussac)

1821 FÉRUSSAC, Hist. Nat. Gén. & Part. Moll. Tabl. Auricules,
  p. 105 (*Scarabus*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
  p. 131–133.

Material examined:

B.   Museum Buitenzorg.

Madoera Id., leg. P. A. OUWENS.

Previous records in literature:

Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 63, p. 222.

Pandjang Id., (near Japara) 1923 OOSTINGH, Meded. Landb.
  Hoogesch. p. 142.

Reported from Ceylon and India, Siam, Borneo, Sumatra and
Java, with the two above-mentioned satellite islands. Madoera
is a new record.

Pythia scarabaeus (Linné)

1758 LINNÉ, Syst. Nat. Ed. X, p. 768 (*Helix*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
  p. 133–136.

Material examined:
    B.   Museum Buitenzorg.
        Sebesi Id., April 1921, leg. K. W. DAMMERMAN.
        Madoera Id., Djoemiang, leg. P. A. OUWENS.
    C.   Museum Leiden.
        Noesa Kembangan, June 1931, leg. W. C. VAN HEURN.
Previous records in literature:
      Krakatau Id. ⎫
      Verlaten Id. ⎬ 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
      Sebesi Id. ⎭   p. 144.
      Krakatau Id. ⎱ 1929 DAMMERMAN, Krakatau's New Fauna,
      Verlaten Id. ⎰   p. 114.
      Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 63, p. 221.
      Krakatau Id. ⎫
      Verlaten Id. ⎬ 1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65,
      Sebesi Id. ⎭   p. 396.

The specimens from Krakatau and Verlaten Ids., mentioned by me in 1925, have to be attributed to *P. chrysostoma*. This holds good also for the references by DAMMERMAN and RENSCH all referring to the same material. The real *P. scarabaeus* has a wide distribution in the Malay Archipelago, especially in the eastern part.

Pythia trigona (Troschel)

1838 TROSCHEL, Arch. Naturgesch. Vol. 1, p. 207, pl. 4, fig. 5 (*Scarabus*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 130–131, pl. 8, fig. 1.

Material examined:
    A.   Museum Amsterdam.
        Noesa Kembangan, 1929, leg. W. G. N. VAN DER SLEEN.

The species is reported from India, Malaya, Sumatra, Borneo, Java and the Philippines. Noesa Kembangan is a new record.

Pythia undata (Lesson)

1831 LESSON, Voy. Coquille, Zool. Vol. 2, p. 336, pl. 10, fig. 6 (*Scarabus*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 139–140.

Material examined:
    B.   Museum Buitenzorg.
        Madoera Id., Tjerek, leg. P. A. OUWENS.

Previous records in literature:

    Madoera Id., 1860 REEVE, Conch. Icon. Vol. 12, *Scarabus*, fig. 27
      (*Scarabus avellana*).

    Madoera Id., 1897 MARTENS, l.c.

    Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol.
      31, p. 247.

    Madoera Id., 1934 RENSCH, Zool. Jahrb. (Syst.) Vol. 65, p. 396.

Common in the entire Malay Archipelago between Sumatra,
and New Guinea, including Java.

Ellobium auris-judae (Linné)

1758 LINNÉ, Syst. Nat. Ed. X, p. 728 (*Bulla*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
    p. 154–158 (*Auricula Judae*).

Material examined:

    A. Museum Amsterdam.

        Middelburg Id., 1938, leg. J. D. F. HARDENBERG.

    B. Museum Buitenzorg.

        Madoera Id., Djoemiang, leg. P. A. OUWENS.

        Madoera Id., Djoemiang and Tjerek, leg. P. A. OUWENS.

Previous records in literature:

    Madoera Id., 1860 ZOLLINGER, Natuurk. Tijdschr. Ned. Indië,
      Vol. 21, p. 316 (*Auricula Indoe*).

    Madoera Id., 1897 MARTENS, l.c.

    Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol.
      31, p. 248 (*Auricula*).

Very common and widely distributed in the Indo-Australian
region from India to North Australia and New Guinea. It is
also frequently recorded from Java.

Ellobium auris-midae (Linné)

1758 LINNÉ, Syst. Nat. Ed. X, p. 728 (*Bulla*).

1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
    p. 150–152 (*Auricula Midae*).

Material examined:

    B. Museum Buitenzorg.

        Kangean Ids., leg. P. A. OUWENS.

    C. Museum Leiden.

        Madoera Id., leg. Mrs. MANGOLD.

Almost equally abundant as the preceding species, but more
confined to the Malay Archipelago, Malaya, Siam and Tonkin.
It is also common in Java. Both the above-mentioned records
are new.

Ellobium subnodosum (Metcalfe)

1851 METCALFE, Proc. Zool. Soc. London, p. 72 (*Auricula*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 152–154 (*Auricula*).

Material examined:
   B. Museum Buitenzorg.
       Madoera Id., Djoemiang and Tjerek, leg. P. A. OUWENS.

The distribution is essentially around the coasts of Borneo and
near Singapore. I have no records from Sumatra. The locality
Java needs further confirmation. Madoera is a new record.

Lymnaea javanica Mousson

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 42–43, pl. 5,
   fig. 1 (*Limnaeus succineus* var. *javanica*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 3, pl. 1, fig. 3–7, pl. 12, fig. 2, 4.

Material examined:
   B. Museum Buitenzorg.
       Madoera Id., Pamekasan, leg. P. A. OUWENS.
   C. Museum Leiden.
       Madoera Id., leg. E. F. JOCHIM.
Previous records in literature:
       Madoera Id., 1867 MARTENS, Malak. Blätt. Vol. 14, p. 224
       (*Limnaeus javanicus* var. d. *rubiginosus*).

The species, with its numerous varieties, is known all over
the Malay Archipelago, including Java.

Anisus (*Gyraulus*) *convexiusculus* (Hutton)

1850 HUTTON, Journ. As. Soc. Bengal. (2) Vol. 18, p. 657
   (*Planorbis*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4,
   p. 13–14, pl. 1, fig. 17–22, pl. 12, fig. 7 and 10 (*Planorbis
   compressus*).

Material examined:
   A. Museum Amsterdam.
       Karimon Djawa Ids., from rivulet near kampong, freshwater,
       May 1926, leg. K. W. DAMMERMAN.
   B. Museum Buitenzorg.
       Madoera Id., Pamekasan, leg. P. A. OUWENS.

The species has a wide distribution, reaching from Mesopo-
tamia, and Persia, via India, Birma, Malaya, China and Japan
to the Philippines, the Indian Archipelago and New Guinea.

Some years ago I discussed the synonymy of *convexiusculus* and *infralineatus* and gave series of localities where it was found in the island of Java. (Treubia, Vol. 13, 1931, p. 5–14) The Karimon Djawa Ids. and Madoera are new records.

Vaginula bleekeri (Keferstein)

1865 KEFERSTEIN, Zeitschr. wiss. Zool. Vol. 15, p. 118, pl. 9,
· fig. 1–7 (*Veronicella*).

1925 HOFFMANN, Jen. Zeitschr. Naturw. Vol. 61, p. 135–137
, (*Vanigula*).

1925 GRIMPE & HOFFMANN, Zeitschr. wiss. Zool. Vol. 124,
p. 31–33.

Material examined: · ⸱
B. Museum Buitenzorg. , ·
Sebesi Id., Sept. 29, 1920, leg. K. W. DAMMERMAN.
· Sebesi Id., April, 25, 1921, leg. K. W. DAMMERMAN.
Previous records in literature: ·
Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143
(*strubelli*).
Sebesi Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 90
(*Vaginula* sp.).

The species is known from Siam, the Anambas and Natoena Ids., Borneo, Sumatra, Sebesi, Java, Amboina and Banda.

Semperula maculata (Templeton)

1858 TEMPLETON, Ann. Mag. Nat. Hist. (3) Vol. 1, p. 49,
pl. 2-B, fig. 1–5 (*Vaginula*).

1925 HOFFMANN, Jen. Zeitschr. Naturw. Vol. 61, p. 258–260.

1925 GRIMPE & HOFFMANN, Zeitschr. wiss. Zool. Vol. 124,
p. 38–44.

Material examined:
· A. Museum Amsterdam.
Hoorn Id., March 1920, leg. K. W. DAMMERMAN.
Previous records in literature:
Noordwachter Id., 1927 HOFFMANN, Ark. f. Zool. Vol. 19–A, p. 37.

A common species in the Indo-Malay region and beyond it. In HOFFMANN and HOFFMANN & GRIMPE (ll.cc.) the localities are mentioned in detail. We have also several references for Java. Hoorn Id. is a new record.

Succinea gracilis Lea

1844 LEA, Proc. Americ. Philos. Soc. Philadelphia, Vol. 2, p. 31.

1867 MARTENS, Ostas. Landschn. p. 387–388.

1932 Van Benthem Jutting, Journ. of Conch. Vol. 19, p. 206.
Material examined:
  A. Museum Amsterdam.
      Noordwachter Id., Sept. 8, 1921, leg. K. W. Dammerman.

There is some uncertainty as to the specific status of *Succinea gracilis* which may be caused by the fact that Lea did not mention any dimensions in his description. As all other characteristics agree perfectly well with the diagnosis I do not hesitate to bring the shells from Noordwachter Id. to this species.

Dimensions of the 10 largest individuals, in mm:

Height	. . . .	8	7.5	8	8	7.5	8	5.5	6	5.5	6
Breadth	. . . .	4.5	5	5	5.5	4.5	5	3.5	4	3.5	4
Height aperture	. . . . .	5.5	5.5	5	5.5	5	5.5	4	4.5	4	4.5
No. of whorls	. . . . . .	3	3	3	3	2.75	3	2.5	2.5	2.25	2.5

The species was confined to Java so far.

Succinea javanica Schepman

1912 Schepman, Proc. malac. Soc. London, Vol. 10, p. 235,
    pl. 10, fig. 12, 13.
1932 Van Benthem Jutting, Journ. of Conch. Vol. 19, p. 206,
    pl. 7, fig. 10, 11.

Material examined:
  A. Museum Amsterdam.
      Krakatau Id., South East part, 100–200 m, March 1, 1931, leg.
      W. M. Docters van Leeuwen.
Previous records in literature:
      Krakatau Id., 1932 Van Benthem Jutting, l.c.

The species is only known from Java, Soemba (Van Benthem Jutting, Treubia, Vol. 10, 1928, p. 158), Lombok (Rensch, Zool. Jahrb. (Syst.) Vol. 63, 1932, p. 125) and Krakatau.

Succinea listeri Smith

1888 Smith, Proc. Zool. Soc. London, p. 537.
1900 Smith, Monogr. Christmas Id., p. 57, pl. 12, 13.
1932 Van Benthem Jutting, Journ. of Conch. Vol. 19, p. 206,
    pl. 7, fig. 12.

Material examined:
  A. Museum Amsterdam.
      Madoera Id., Pamekasan, P. A. Ouwens.
Previous records in literature:
      Madoera Id., 1932 Van Benthem Jutting, l.c.

Only known from Christmas Id. and Madoera, and, recently, from Java (specimens in Museum Amsterdam, collected in sawah near Kapoek, near Batavia, Aug. 8, 1938, leg. J. KNOCK).

Succinea obesa Martens

1867 MARTENS, Ostas. Landschn. p. 387, pl. 22, fig. 21.
1932 VAN BENTHEM JUTTING, Journ. of Conch. Vol. 19, p. 207, pl. 7, fig. 13.

Material examined:
    A. Museum Amsterdam.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.
Previous records in literature:
        Madoera Id., 1932 VAN BENTHEM JUTTING, l.c.

The species is known from Java, Madoera and Sumatra (RENSCH, Trop. Binnengew. Vol. 4, 1934, p. 757).

Tornatellina cylindrica Sykes

1901 SYKES, Fauna Hawaiiensis, Moll. p. 381, pl. 9, fig. 28.
1915 PILSBRY, Man. of Conch. (2) Vol. 23, p. 153–154, pl. 43, fig. 1, 2, 3, pl. 40, fig. 1, 2.

Material examined:
    A. Museum Amsterdam.
        Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.

The species was only known from the Hawaiian Islands.

Tornatellina subcylindrica Quadras & Möllendorff

1894 QUADRAS & MÖLLENDORFF, Nachr. Blatt, Vol. 26, p. 16.
1915 PILSBRY, Man. of Conch. (2) Vol. 23, p. 166, pl. 33, fig. 10–11.

Material examined:
    A. Museum Amsterdam.
        Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
        Zuidwachter Id., Oct. 22, 1921, leg. K. W. DAMMERMAN.

The two samples, collected by DAMMERMAN, agree in every respect with shells from the Marianne Ids. in the Rijksmuseum van Natuurlijke Historie at Leiden, purchased in 1906 from H. B. PRESTON.

The species was only known from Guam, Marianne Archipelago.

Gastrocopta (*Cavipupa*) *euryomphala* Pilsbry

1898 MÖLLENDORFF, Abh. naturf. Ges. Görlitz, Vol. 22, p. 153 (*Leucochilus euryomphalum* nomen nudum).
1917 PILSBRY, Man. of Conch. (2) Vol. 24, p. 141 (name only).
1934 PILSBRY, Man. of Conch. (2) Vol. 28, p. 120, pl. 22, fig. 3–6.
1937 HAAS, Arch. Moll. Kunde, Vol. 69, p. 3–4, fig. 3–4.

Material examined:
  A. Museum Amsterdam:
      Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
      Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.
      Klein Kombuis Id., July 21, 1922, leg. K. W. DAMMERMAN.
      Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.

The specimens before me are well developed and sufficiently typical. In some of them the costulations on the last whorl are obsolete so as to become almost invisible. This may be a matter of deterioration.

Gastrocopta euryomphala was only known from the Philippines so far.

Gastrocopta lyonsiana (Ancey)

1892 ANCEY, Mém. Soc. Zool. France, Vol. 5, p. 713 (*Pupa*).
1917 PILSBRY, Man. of Conch. (2) Vol. 24, p. 141–144, pl. 24, fig. 1–4.

Material examined:
  A. Museum Amsterdam.
      Klein Kombuis Id., July 21, 1922, leg. K. W. DAMMERMAN.
      Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.

The shells agree exactly with pl. 24, fig. 3 in Man. of Conch. (2) Vol. 24, 1917, all possessing the basal fold. Their habitus differs markedly from *Gastrocopta pediculus* and allied forms, although the points of divergence are difficultly put into words.

The species was not recorded in the Malay Archipelago before. Its area of distribution occupied the Hawaiian Islands and the Philippines so far.

Gastrocopta pediculus ovatula (Möllendorff)

1890 MÖLLENDORFF, Ber. Senck. naturf. Ges. 1890, p. 253 (*Leucochilus pediculus* var. *ovatula*).
1917 PILSBRY, Man. of Conch. (2) Vol. 24, p. 149, pl. 25, fig. 13, 15.

Material examined:
  A. Museum Amsterdam.
      Krakatau Id., Eastern part, soil fauna, Chemara wood, April 24,
        1920, leg. K. W. DAMMERMAN.
      Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
      Zuidwachter Id., Oct. 22, 1921, leg. K. W. DAMMERMAN.
      Klein Kombuis Id., Nov. 11, 1920. leg. K. W. DAMMERMAN.
      Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.
  B. Museum Buitenzorg.
      Krakatau Id., Eastern part, soil fauna, Chemara wood, April 24,
        1920, leg. K. W. DAMMERMAN.
      Verlaten Id., Aug. 1930, leg. K. W. DAMMERMAN.
Previous records in literature:
      Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p.
        140, 143, fig. 1 (*Nesopupa* cf. *micra*).
      Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 114
        (*Nesopupa* sp.).

It was PILSBRY (Man. of Conch. (2) Vol. 27, p. 226, 1926) who
suggested that the Krakatau shells might belong to *Gastrocopta*.
  The subspecies is distributed in the Philippines, Caroline Ids.,
Soemba, and Timor. It will certainly prove to have a much
wider dispersal in our Archipelago. Other subspecies of *Gastro-
copta pediculus* occur in SE Australia, several Polynesian Ids.,
Banda group and Aroe Ids.

Ena (Coccoderma) glandula (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part. 3,
      p. 266 (*Bulimus*).
1849 MOUSSON, Land- & Süssw. Moll. Java, p. 34–35, pl. 4,
      fig. 3 (*Bulimus*).
1867 MARTENS, Ostas. Landschn. p. 370 (*Buliminus*).

Previous records in literature:
      Madoera Id., 1867 MARTENS, l.c.
      Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol.
        31, p. 236 (*Buliminus*).
      Madoera Id., 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 155
        (*Buliminus*).

The species is only known from Java and Madoera.

Hemiphaedusa (Acrophaedusa) cornea (Philippi)

1846 PHILIPPI, in: PFEIFFER, Symb. Vol. 3, p. 63 (*Clausilia*)
      (nomen nudum).
1847 PHILIPPI, in: KÜSTER, in: MART.-CHEMN. N. Syst. Conch.
      Cab. Vol. 1, Part 14, p. 22–23, pl. 2, fig. 1–4 (*Clausilia*).
1867 MARTENS, Ostas. Landschn. p. 383 (*Clausilia*).

Material examined:
    A. Museum Amsterdam
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

The species was only known from Java. Madoera is a new record.

Phaedusa (Pseudonenia) corticina (Von dem Busch)

1842 VON DEM BUSCH, in: PFEIFFER, Symb., Vol. 2, p. 60 (Clausilia).
1867 MARTENS, Ostas. Landschn. p. 381 (Clausilia).

Material examined:
    A. Museum Amsterdam.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

The species is known from Java and Sumatra. Madoera is a new record.

Phaedusa (Pseudonenia) heldi (Küster)

1846 KÜSTER, in: PFEIFFER, Symb. Vol. 3, p. 63 (Clausilia) (nomen nudum).
1847 KÜSTER, in: MART.-CHEMN. N. Syst. Conch. Cab. Vol. 1, Part 14. p. 27–28, pl. 2, fig. 29–31 (Clausilia).
1867 MARTENS, Ostas. Landschn. p. 380–381 (Clausilia).

Material examined:
    A. Museum Amsterdam.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.

Phaedusa heldi was only known from Java so far. Madoera is a new record. BOETTGER (Ber. Senckenb. naturf. Ges. 1890, p. 148) considers *Ph. heldi* a synonym of *Ph. javana* (Pfr.).

Phaedusa (Pseudonenia) heldi var. baronensis (Mousson)

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 39–40, pl. 4, fig. 7 (Clausilia).
1867 MARTENS, Ostas. Landschn. p. 381 (Clausilia).

Previous records in literature:
        Noesa Baron, 1849 MOUSSON, l.c.
        Noesa Baron, 1867 MARTENS, l.c.
        Noesa Baron, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 239.
        Noesa Baron, 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11, p. 81.

The variety is specific to Noesa Baron.

Phaedusa (Pseudonenia) javana (Pfeiffer)

1841 PFEIFFER, Symb. Vol. 1, p. 49 (*Clausilia*).
1867 MARTENS, Ostas. Landschn. p. 380 (*Clausilia*).

Material examined:
A. Museum Amsterdam.
    Noesa Kembangan, June 1931, leg. Mrs. A. C. VAN HEURN.
    Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN
    LEEUWEN.

The species was only known from Java. Noesa Kembangan is
a new record.

Phaedusa (Pseudonenia) moritzi (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 267
(*Clausilia*).
1849 MOUSSON, Land- & Süssw. Moll. Java, p. 41, pl. 4, fig. 8
(*Clausilia*).
1867 MARTENS, Ostas. Landschn. p. 383 (*Clausilia*).

Previous records in literature:
    Noesa Baron, 1849 MOUSSON, Land- & Süssw. Moll. Java, p. 41
    (*Clausilia*).
    Noesa Baron, 1867 MARTENS, l.c.
    Noesa Baron, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg. Vol.
    31, p. 239.

The species is only known from Noesa Baron so far. COOKE
(Proc. malac. Soc. London, Vol. 11, 1915, p. 265) regarded
Phaedusa moritzi as a variety of *Ph. heldi* (Küst.).

Phaedusa (Pseudonenia) sumatrana (Martens)

1864 MARTENS, Monatsber. Akad. Berlin, p. 270 (*Clausilia*).
1867 MARTENS, Ostas. Landschn. p. 379, pl. 22, fig. 17 (*Clausilia*)

Material examined:
B. Museum Buitenzorg.
    Sebesi Id., April 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
    Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143.

Only known from Sumatra and Sebesi.

Subulina octona (Bruguière)

1789 BRUGUIÈRE, Encycl. Méth. (Vers) Vol. 1, p. 325 (*Bulimus*).
1890 BOETTGER, Ber. Senckenb. naturf. Ges. p. 147.

Material examined:
A. Museum Amsterdam.
> Krakatau Id., in detritus, in jungle, May 13, 1929, leg. L. F. DE BEAUFORT.
> Noordwachter Id., Sept. 1921, leg. H. BOSCHMA.
B. Museum Buitenzorg.
> Krakatau Id., May 1928, leg. W. M. DOCTERS VAN LEEUWEN.
> Krakatau Id., soil fauna, Jan. 1933, leg. K. W. DAMMERMAN.
> Krakatau Id., soil fauna, April 1933, leg. K. W. DAMMERMAN.
C. Museum Leiden.
> Noordwachter Id., Nov. 2–8, 1921, leg. L. DE PRIESTER.

Very common in the moss-fauna of several islands in the Malay Archipelago. The first record for Java dates from 1890 (BOETTGER, l.c.). Krakatau and Noordwachter are new localities.

Opeas gracile (Hutton)

1834 HUTTON, Journ. As. Soc. Bengal, Vol. 3, p. 93 (*Bulimus*).
1867 MARTENS, Ostas. Landschn. p. 376 (*Stenogyra*).

Material examined:
A. Museum Amsterdam.
> Prinsen Id., soil fauna, Jan. 1922, leg. K. W. DAMMERMAN.
> Krakatau Id., soil fauna, April 24, 1920, leg. K. W. DAMMERMAN.
> Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
> Zuidwachter Id., Oct. 22, 1921, leg. K. W. DAMMERMAN.
> Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.
> Klein Kombuis Id., July 21, 1922, leg. K. W. DAMMERMAN.
> Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.
> Karimon Djawa Ids., soil fauna, May 1926, leg. K. W. DAMMERMAN.
> Karimon Djawa Ids., under pieces or coral, circa 300 m from the shore, Nov. 24, 1930, leg. M. A. LIEFTINCK.
> Karimon Djawa Ids., Poeloe Kemoedjan, under leaves and stones, in cocoanut garden, behind the shore, Nov. 30, 1930, leg. M. A. LIEFTINCK.
B. Museum Buitenzorg.
> Krakatau Id., soil fauna, April 24, 1920, leg. K. W. DAMMERMAN.
> Krakatau Id., South East part, 100–200 m, March 1, 1931, leg. W. M. DOCTERS VAN LEEUWEN.
> Krakatau Id., soil fauna, Jan. 1933, leg. K. W. DAMMERMAN.
> Verlaten Id., soil fauna, Oct. 24, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
> Madoera Id., 1849 MOUSSON, Zeitschr. Malak. Vol. 6, p. 180 (*Bulimus apex*).
> Madoera Id., 1867 MARTENS, l.c.
> Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamb. Vol. 31, p. 238.

20

Krakatau Id., ⎱ 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
Verlaten Id., ⎰   p. 143.
Krakatau Id., ⎱ 1929 DAMMERMAN, Krakatau's New Fauna,
Verlaten Id., ⎰   p. 114.

According to H. BURRINGTON BAKER (Nautilus, Vol. 48, 1935, p. 84) the species has to be called *Lamellaxis* (*Allopeas*) *gracilis* (Hutton). It is a tropical cosmopolite.

Prosopeas achatinaceum (Pfeiffer)

1846 PFEIFFER, Symb. Vol. 3, p. 82 (*Bulimus*).
1867 MARTENS, Ostas. Landschn. p. 375, pl. 22, fig. 9 (*Stenogyra*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 113–115.

Material examined:
    A.   Museum Amsterdam.
          Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.
          Bawean Id., South part, under stones, May 1928, leg. K. W. DAMMERMAN.
          Bawean Id., South part, soil fauna, May 1928, leg. K. W. DAMMERMAN.
          Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
    B.   Museum Buitenzorg.
          Sebesi Id., soil fauna, May 26, 1921, leg. K. W. DAMMERMAN.
          Sebesi Id., soil fauna, Oct. 26, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
          Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143.

Inhabiting Borneo, Sumatra, Java, Sebesi, Bali, Lombok, Timor and Saleyer in the Malay Archipelago and the Caroline Ids. beyond it. Bawean and Noesa Kembangan are new records.

Prosopeas turriculum (Martens)

1867 MARTENS, Ostas. Landschn. p. 82–83, pl. 22, fig. 7 (*Stenogyra*).

Material examined:
    A.   Museum Amsterdam.
          Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.
    B.   Museum Buitenzorg.
          Sebesi Id., Sept. 29, 1920, leg. K. W. DAMMERMAN.
          Sebesi Id., April 1921, leg. K. W. DAMMERMAN.
          Sebesi Id., soil fauna, leg. K. W. DAMMERMAN.
Previous records in literature:
          Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143.
          Sebesi Id., 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11, p. 80.

The species is known from Siam, Java and Sebesi.

Stenopylis coarctata (Möllendorff) (fig. 2 and 3)

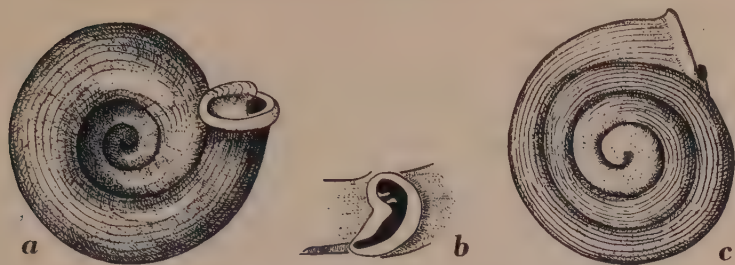1894 MÖLLENDORFF, Nachr. Blatt, Vol. 26, p. 113 (*Plectopylis*).

Fig. 2. *Stenopylis coarctata* (Möllendorff). A. base view, B. aperture, C. dorsal view, x 20. Enkhuizen Id., March 18, 1920.

1914 FULTON, Ann. Mag. Nat. Hist. (8) Vol. 14, p. 163.
1915 FULTON, Proc. malac. Soc. London, Vol. 11, p. 236.
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 103.
1935 RENSCH, Sitz. Ber. Ges. naturf. Freunde, Berlin, p. 322.

Material examined:
    A. Museum Amsterdam.
        Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
        Zuidwachter Id., Oct. 22, 1921, leg. K. W. DAMMERMAN.
        Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.
        Klein Kombuis Id., Aug. 21, 1922, leg. K. W. DAMMERMAN.
        Enkhuizen Id., March 18, 1920, leg. K. W. DAMMERMAN.
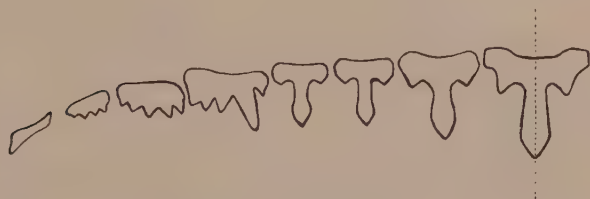
Fig. 3. *Stenopylis coarctata* (Möllendorff). Half a row of teeth from radula. Zuidwachter Id., Oct. 22, 1921.

The species is known from the Philippines, Soemba, Timor, New Guinea and Central Australia (RENSCH, ll.cc.). The above-mentioned islands are new records.

Kaliella doliolum (Pfeiffer)

1846 PFEIFFER, Proc. Zool. Soc. London, p. 41 (*Helix*).
1934 RENSCH, Trop. Binnengew. Vol. 4, p. 749–750.

Material examined:
  A. Museum Amsterdam.
          Krakatau Id., soil fauna, Jan. 1933, leg. K. W. DAMMERMAN.
          Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
          Zuidwachter Id., Oct. 22, 1921, leg. K. W. DAMMERMAN.
          Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.
          Klein Kombuis Id., Jan. 22, 1922, leg. K. W. DAMMERMAN.
          Klein Kombuis Id., July 21, 1922, leg. K. W. DAMMERMAN.
          Edam Id., Nov. 27, 1919, leg. K. W. DAMMERMAN.
          Karimon Djawa Ids., Poeloe Kemoedjan, under leaves and
            stones in coconut garden close to the shore, Nov. 30, 1930, leg.
            M. A. LIEFTINCK.
  B. Museum Buitenzorg.
          Krakatau Id., soil fauna, April 1934, leg. K. W. DAMMERMAN.

Several specimens from Klein Kombuis Id., Nov. 11, 1920,
carry eggs and pulli which are shining through the shell, thus
demonstrating that the species is ovoviviparous.

RENSCH (l.c.) gave a list of all the islands where *K. doliolum*
was observed. The above-mentioned islands are all new localities.
Besides it was mentioned from Borneo (MARTENS, Mitt. Zool.
Mus. Berlin, Vol. 4, 1908, p. 261) and I have records from the
Malay Peninsula (VAN BENTHEM JUTTING, Bull. Raffles Mus.
Singapore, in the press), Berhala Id. and the Kei Ids., all in
Museum Amsterdam.

Kaliella indifferens Boettger

1891 BOETTGER, Ber. Senck. naturf. Ges. p. 256–257, pl. 3, fig. 4.
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 62–63.

Material examined:
  A. Museum Amsterdam.
          Krakatau Id., soil fauna, Dec. 12, 1919, leg. K. W. DAMMERMAN.
          Krakatau Id., South East part, 100–200 m alt., March 1, 1931,
            leg. W. M. DOCTERS VAN LEEUWEN.
          Verlaten Id., soil fauna, Jan. 1933, leg. K. W. DAMMERMAN.
          Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.
  B. Museum Buitenzorg.
          Krakatau Id., soil fauna, Dec. 12, 1919, leg. K. W. DAMMERMAN.
          Verlaten Id., soil fauna, Oct. 24, 1921, leg. K. W. DAMMERMAN.
          Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
          Krakatau Id., }
          Verlaten Id., } 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
          Sebesi Id.,   }   p. 143.

          Krakatau Id., } 1929 DAMMERMAN, Krakatau's New Fauna,
          Verlaten Id., }   p. 114.

Krakatau Id., |
Verlaten Id., } 1932 RENSCH, l.c.
Sebesi Id., |

Krakatau Id., |
Verlaten Id., | 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 749.

RENSCH (l.c., 1932) gives a list of all the islands where *Kaliella indifferens* was found. There are no new records since that time.

Trochomorpha (Videna) bicolor Martens

1864 MARTENS, Monatsber. Akad. Berlin, p. 267.
1867 MARTENS, Ostas. Landschn. p. 252–253, pl. 13, fig. 2.
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 77–78.

Previous records in literature:
      Dwars in den Weg Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56.

To the list of all the islands where *Tr. bicolor* was found (RENSCH, l.c.) the little island Dwars in den Weg has to be added.

Trochomorpha (Videna) planorbis (Lesson)

1831 LESSON, Voy. Coquille, Zool. Vol. 2, p. 312, pl. 13, fig. 4 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 249, pl. 13, fig. 4, 7, 8.

Material examined:
  A. Museum Amsterdam.
      Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1927, leg. P. F. FRANCK.

The species is reported from several islands in the Malay Archipelago, including Java, and equally from the Philippines and New Pommerania (RENSCH, Zool. Jahrb. (Syst.) Vol. 63, 1932, p. 79). Noesa Kembangan is a new record.

Eurybasis (Chiroktisma) conus (Philippi)

1841 PHILIPPI, in: PFEIFFER, Symb. Vol. 1, p. 39 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 253 (*Trochomorpha*).
1932 VAN BENTHEM JUTTING, Journ. of Conch. Vol. 19, p. 208–209, pl. 7, fig. 14 and 16 (*Chiroktisma*).

Material examined:
  B. Museum Buitenzorg.
      Madoera Id., Pegantenan, leg. P. A. OUWENS.
Previous records in literature:
      Noesa Baron, 1849 MOUSSON, Land- & Süssw. Moll. Java, p. 20, pl. 2, fig. 2 (*Helix*).
      Noesa Baron, 1867 MARTENS, l.c.

Noesa Baron, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol.
31, p. 228 (*Trochonanina*).
Noesa Baron, 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11,
p. 77 (*Chiroktisma*).
Madoera Id., 1932 VAN BENTHEM JUTTING, l.c.

The species is known from West Java, Noesa Baron and
Madoera. I my paper (l.c., 1932) I expressed already the opinion
that the latter locality needs further confirmation.

Eurybasis (Chiroktisma) multicarinata (Boettger)

1890 BOETTGER, Ber. Senck. naturf. Ges. p. 141 (*Trochonanina*).
1932 VAN BENTHEM JUTTING, Journ. of Conch. Vol. 19, p.
208–210, pl. 7, fig. 15 (*Chiroktisma*).

Material examined:
B. Museum Buitenzorg.
Madoera Id., Pegantenan, leg. P. A. OUWENS.
Previous records in literature:
Madoera Id., 1932 VAN BENTHEM JUTTING, l.c.

The species is confined to West Java and Madoera, the latter
locality, however, with a certain amount of doubt (VAN BENTHEM
JUTTING, l.c.).

a   *b*

Fig. 4. *Microcystina nana* (Möllendorff). A. base
view, B. dorsal view, x 12. P. Kemoedjan,
Karimon Djawa Ids., Nov. 30, 1930.

Microcystina nana (Möllendorff) (fig. 4)

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 63 (*Lamprocystis*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 48–49, fig. 12.

Material examined:
A. Museum Amsterdam.
Karimon Djawa Ids., Poeloe Kemoedjan, under leaves and stones
in coconut garden, close to the shore, Nov. 30, 1930, leg.
M. A. LIEFTINCK.

The species was known from Java, Bali and Flores (RENSCH, l.c.). The Karimon Djawa Ids. form a new record. In all previous instances *Microcystina nana* was found in mountainous region, at an altitude of 500 m or more. In the Karimon Djawa Ids., however, 4 shells were collected alive almost at sea-level in a coconut plantation at a short distance behind the beach.

Hemiplecta bataviana (Von dem Busch)

1842 VON DEM BUSCH, in: PFEIFFER, Symb. Vol. 2, p. 17 (*Helix*).
1856 PFEIFFER, Proc. Zool. Soc. London, p. 327 (*Helix arguta*).
1867 MARTENS, Ostas. Landschn. p. 217–219 (*Nanina*), p. 219–220 (*Nanina arguta*).
1934 RENSCH, Trop. Binnengew. Vol. 4, p. 746–747.

Material examined:
    A. Museum Amsterdam.
        Meeuwen Id., July 23, 1924, leg. K. W. DAMMERMAN.
        Meeuwen Id., March 30, 1932, leg. P. F. FRANCK.
        Bawean Id., South part, May 1928, leg. K. W. DAMMERMAN.
        Madoera Id., Pamekasan, leg. P. A. OUWENS.
        Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.
    C. Museum Leiden.
        Bawean Id., leg. DIARD.
Previous records in literature:
        Krakatau Id., 1867 MARTENS, l.c. (*Nanina arguta*).
        Krakatau Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (*arguta*).
        Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 142 (*arguta*).
        Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 86 (*Hemiplecta* sp.).
        Krakatau Id., / 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 747.
        Bawean Id.,   \
        Bawean Id., 1867 MARTENS, l.c. (*Nanina*).
        Bawean Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56.
        Bawean Id., 1936 RENSCH, Gesch. Sunda Bogens, p. 110.

The entries for Krakatau are all referring to a find from before the eruption of 1883. After that catastrophe the species is no longer living in the island. It is a common inhabitant of Java, Sumatra and Borneo.

Hemiplecta bataviana var. *duplocincta* (Möllendorff)

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 64 (*Ariophanta duplocincta*).

Material examined:
  A. Museum Amsterdam.
      Bawean Id., North part, June 2, 1920, leg. K. W. DAMMERMAN.
      Bawean Id., South part, May 1928, leg. K. W. DAMMERMAN.

The variety is living side by side with the main form. Therefore some authors do not credit it varietal rank and simply insert it in *Hemiplecta bataviana* (RENSCH, Trop. Binnengew. Vol. 4, 1934, p. 747).

Hemiplecta humphreysiana (Lea)

1841 LEA, Trans. Americ. Philos. Soc. Philadelphia, Vol. 7, p. 463, pl. 12, fig. 16 (*Helix*).

1867 MARTENS, Ostas. Landschn. p. 233, pl. 10, fig. 2, 2-b, 4 (*Nanina*).

1934 RENSCH, Arch. Moll. Kunde, Vol. 66, p. 322–327.

Material examined:
  A. Museum Amsterdam.
      Meeuwen Id., March 30, 1932, leg. P. F. FRANCK.
      Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1929, leg. P. F. FRANCK.
Previous records in literature:
      Noesa Kembangan, 1912 SCHEPMAN, Proc. malac. Soc. London, Vol. 10, p. 230–231.
      Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 226.
      Noesa Kembangan, 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 150.

The shell from Noesa Kembangan, collected by Mr. FRANCK, belongs to the race *densa* (Ads. 1850). For the distribution of the species I must refer to the paper of RENSCH (l.c.).

Hemiplecta javacensis (Férussac)

1821 FÉRUSSAC, Hist. Nat. génér. & part. Moll. Tabl. Limaçons, p. 46 (*Helix*).

1842 GUILLOU, Rev. Zool. p. 137 (*Helix umbilicaria*).

1867 MARTENS, Ostas. Landschn. p. 214–215 (*Nanina umbilicaria*), p. 215–217, pl. 6, fig. 5 (*Nanina Javana*).

1934 RENSCH, Trop. Binnengew. Vol. 4, p. 746.

Material examined:
  A. Museum Amsterdam.
      Karimon Djawa Ids., Oct. 1920, leg. H. C. DELSMAN.
      Karimon Djawa Ids., May 1926, leg. K. W. DAMMERMAN.
      Karimon Djawa Ids., Wood near the churchyard, Nov. 23, 1930, leg. M. A. LIEFTINCK.

    Karimon Djawa Ids., Poeloe Kemoedjan, Nov. 25, 1930, leg.
     M. A. LIEFTINCK.
    Madoera Id., leg. P. A. OUWENS.
    Madoera Id., Dec. 8, 1914, leg. P. A. OUWENS.
    Madoera Id., leg. SANGSTER.
  B. Museum Buitenzorg.
    Madoera Id., Djoemiang, leg. P. A. OUWENS.
Previous records in literature:
    Krakatau Id., 1867 MARTENS, l.c. (*Nanina umbilicaria* var. *suma-*
     trana).
    Krakatau Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (*um-*
     bilicaria var. *sumatrana*).
    Krakatau Id., 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 148.
    Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
     p. 142 (*umbilicaria* var. *sumatrana*).
    Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 86
     (*Hemiplecta* sp.).
    Krakatau Id., 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 746.
    Madoera Id., 1867 MARTENS, l.c. (*Nanina Javana*).
    Madoera Id., 1903 GUDE, l.c. (*javana*).
    Madoera Id., ⎰ 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67,
    Kangean Ids., ⎱   p. 172 (*javana*).

The references to Krakatau all refer to material from before
the eruption of 1883. After this catastrophe the species has not
settled there again.

So far the distribution of *Hemiplecta javacensis* included Sumatra,
Banka, Java, Madoera and Kangean Ids., the Karimon Djawa
group is a new habitat.

<center>*Hemiplecta kangeanensis* Schepman</center>

1913 SCHEPMAN, Siboga Exp. Monogr. 49–1–f, p. 455, pl. 31,
  fig. 1.

Material examined:
  A. Museum Amsterdam.
    Kangean Ids., Siboga Expedition.
Previous records in literature:
    Kangean Ids., 1913 SCHEPMAN, l.c.

So far the species is confined to the Kangean Islands.

<center>*Hemiplecta virens* Martens</center>

1867 MARTENS, Ostas. Landschn. p. 237 (*Nanina*).
1880 MARTENS, Conch. Mitt. Vol. 1, p. 2–3, pl. 1, fig. 4–6 (*Nanina*).

Previous records in literature:
    Dwars in den Weg Id., 1867 MARTENS, l.c.
    Dwars in den Weg Id., 1880 MARTENS, l.c.
    Dwars in den Weg Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56.

Only recorded from Sumatra and Dwars in den Weg. I do not know the species.

Dyakia martini (Pfeiffer)

1854 PFEIFFER, Proc. Zool. Soc. London, p. 149 (*Helix*).
1864 MARTENS, Monatsber. Akad. Berlin, p. 265 (*Nanina*).
1867 MARTENS, Ostas. Landschn. p. 221, pl. 11, fig. 2, 5, pl. 6, fig. 3, 3-b (*Nanina amphidroma* var. *martini*).
1934 RENSCH, Arch. Moll. Kunde, Vol. 66, p. 327–329.

Previous records in literature:
      Dwars in den Weg Id., 1867 MARTENS, l.c.
      Dwars in den Weg Id., 1874 ISSEL, Ann. Mus. Civ. St. Nat. Genova, Vol. 6, p. 394 (*Nanina amphidroma*).
      Dwars in den Weg Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (*Dyakia amphidroma* var. *martini*).

ISSEL (l.c.) mentioned the islands Sungian and Dwars (= Dwars in den Weg) in Soenda Strait as 2 separate localities. The question is that they are one and the same island, Soengian (or Sungian) being the native name of Dwars in den Weg.

Dyakia martini is known from the Malay Peninsula, Borneo, Sumatra, Banka, and Dwars in den Weg. So far we have no records from Java.

Dyakia rumphii (Von dem Busch)

1842 VON DEM BUSCH, in: PFEIFFER, Symb. Vol. 2, p. 20 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 220–221 (*Nanina*).
1934 RENSCH, Trop. Binnengew. Vol. 4, p. 748.

Material examined:
  B. Museum Buitenzorg.
      Madoera Id., Pegantenan, leg. P. A. OUWENS.
      Madoera Id., Pamekasan, leg. P. A. OUWENS.

A common jungle snail in Sumatra and Java. Madoera is a new record.

Durgellina convexoconica (Möllendorff)

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 60 (*Kaliella*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 67–68 (*Durgellina?*).
1934 RENSCH, Trop. Binnengew. Vol. 4, p. 750.

Material examined:
  A. Museum Amsterdam.
      Krakatau Id., soil fauna, April 1933, leg. K. W. DAMMERMAN.
      Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.

B   Museum Buitenzorg
            Krakatau Id , soil fauna, April 1933, leg. K. W. DAMMERMAN.
            Sebesi Id., Oct., 26, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
            Sebesi Id., 1925 VAN BENTHEM JUTTING. Treubia, Vol. 6, p. 143
              (*Kaliella*).
            Sebesi Id., 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11, p. 78
              (*Kaliella*).
            Sebesi Id., 1932 RENSCH, l.c.
            Sebesi Id., 1934 RENSCH, l.c.

The species is known from Java, Sebesi and Soemba. Krakatau
is a new record.

Helicarion adolfi Boettger

1890 BOETTGER, Ber. Senck. naturf. Ges. p. 138-139, pl. 5,
      fig. 1-*a*, *b*, *c*.
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 71–72, fig. 25.
Material examined:
    A. Museum Amsterdam.
            Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN
            LEEUWEN.

The species was known from Sumatra, Java, Bali, Lombok
and South Celebes (RENSCH, Trop. Binnengew. Vol. 4, 1934,
p. 753). Noesa Kembangan is a new record.

Helicarion lineolatus Martens

1867 MARTENS, Ostas. Landschn. p. 184, pl. 12, fig. 4.
Material examined:
    A. Museum Amsterdam.
            Sebesi Id., 700 m alt., April 25, 1921, leg. K. W. DAMMERMAN.
    B. Museum Buitenzorg.
            Sebesi Id., April 25, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
            Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143.
            Sebesi Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 90
              (*Helicarion* sp.).

The species is only known from Sumatra and Sebesi.

Microparmarion (*Collingea*) *strubelli* Simroth

1893 SIMROTH, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 3,
      p. 108–109, pl. 7, fig. 5 and 11, pl. 8, fig. 12 and 15.

Material examined:
    A. Museum Amsterdam.
            Sebesi Id., 700 m alt., April 25, 1921, leg. K. W. DAMMERMAN.

B. Museum Buitenzorg.
    Sebesi Id., April 25, 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
    Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143
    (*Collingea*).
    Sebesi Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 90
    (*Collingea* sp.)

The species is only known from Java and Sebesi.

Landouria ciliocincta (Möllendorff)

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 67 (*Plectotropis*).

Material examined:
   A. Museum Amsterdam.
    Small island off Karang Anjer, West of Semarang, July 5, 1937,
    leg. G. M. VAN REGTEREN ALTENA.
    Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1927,
    leg. P. F. FRANCK.
    Noesa Kembangan, 1929, leg. W. G. N. VAN DER SLEEN.

The specimens from Noesa Kembangan are typical in form and structure, quite fresh and beautifully displaying the rufous peripheral band. The two shells from the minuscule island off Karang Anjer are weathered and bleached. The species was known from West and Central Java so far. The above-mentioned localities extend its area of distribution to the North and to the South.

Landouria kangeanensis (Schepman)

1913 SCHEPMAN, Siboga Exp. Monogr. 49-1-*f*, p. 456, pl. 31, fig. 2 (*Plectotropis*).

Material examined:
   A. Museum Amsterdam.
    Kangean Ids., Siboga Expedition.
Previous records in literature:
    Kangean Ids., 1913 SCHEPMAN, l.c.

The species is only known from the Kangean Islands so far.

Landouria rotatoria (Von dem Busch)

1842 VON DEM BUSCH, in: PHILIPPI, Abb. Conch. Vol. 1, p. 2, pl. 1, fig. 5 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 264 (*Helix*).
1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 211–212, fig. 5–7 (*Plectotropis kraepelini*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 91–93.

Material examined:
  A. Museum Amsterdam.
          Noesa Kembangan, Nature Reserve Gligir, Febr. 15–16, 1927,
              leg. P. F. FRANCK.
          Noesa Kembangan, March 1932, Leg. W. M. DOCTERS VAN
              LEEUWEN.
  B. Museum Buitenzorg.
          Sebesi Id., Oct. 26, 1921, leg. K. W. DAMMERMAN.
          Sebesi Id., Oct. 27, 1921, leg. K. W. DAMMERMAN.
          Sebesi Id., Jan. 1922, leg. K. W. DAMMERMAN.
          Madoera Id., Pegantenan, leg. P. A. OUWENS.
Previous records in literature:
          Popole Id., 1849 MOUSSON, Land- & Süssw. Moll. Java, p. 25,
              pl. 2, fig. 8 (Helix).
          Popole Id., 1867 MARTENS, l.c.
          Popole Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (Plecto-
              tropis).
          Popole Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg. Vol. 31,
              p. 235 (Plectotropis).
          Sebesi Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 143
              (Plectotropis kraepelini).
          Sebesi Id., 1929 VAN BENTHEM JUTTING, Treubia, Vol. 11, p.
              80 (Plectotropis kraepelini).
          Sebesi Id., 1931 RENSCH, Zool. Jahrb. (Syst.) Vol. 60, p. 445.
          Sebesi Id., 1932 RENSCH, l.c.
          Sebesi Id., 1933 RENSCH, Sitz. Ber. Ges. Naturf. Freunde Berlin,
              p. 507.
          Sebesi Id., 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 754.
          Sebesi Id., 1935 RENSCH, Sitz. Ber. Ges. naturf. Freunde Berlin,
              p. 335.
          Kangean Ids., 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67,
              p. 173.

The species is known from Sebesi, Popole, Java, Kangean,
Bali, Lombok, Soembawa, Flores, Timor, and the Philippines.
Madoera and Noesa Kembangan are new records.

Landouria squamulosa (Martens)

1867 MARTENS, Ostas. Landschn. p. 266–267 (Helix).

Previous records in literature:
          Madoera Id., 1867 MARTENS, l.c.
          Madoera Id., 1888 TENISON WOODS, Proc. Linn. Soc. N. S. Wales
              (2) Vol. 3, p. 1032 (Helix (Plectotropis)).
          Madoera Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56
              (Plectotropis).

Landouria squamulosa is only recorded from Madoera. I do not
know the species.

Landouria winteriana (Pfeiffer)

1842 PFEIFFER, Symb. Vol. 2, p. 41 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 264, pl. 13, fig. 11 (*Helix*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 88–90, fig. 34, 35.

Material examined:
    A. Museum Amsterdam.
        Klapper Id., Febr. 18–23, 1932, leg. DARNA and SAÄN.

The species is a common inhabitant of Java. Beyond this island it is known from Siam, Borneo, Sumatra, Flores, Soemba, Timor, Celebes, Halmahera, Ternate, Batjan and the Philippines. Klapper Id. is a new locality.

Chloritis crassula (Philippi)

1844 PHILIPPI, Abb. Conch. Vol. 1, p. 152 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 276 (*Helix*).

Previous records in literature:
        Noesa Kembangan, 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67
        p. 172.

The species is only known from Nias, Java and Noesa Kembangan.

Chloritis fruhstorferi Möllendorff

1897 MÖLLENDORFF, Nachr. Blatt, Vol. 29, p. 68.

Material examined:
    A. Museum Amsterdam.
        Noesa Kembangan, June 1931, leg. Mrs. A. C. VAN HEURN.
        Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN
        LEEUWEN.

The species was only known from Java so far. Noesa Kembangan is a new record.

Chloritis helicinoides (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 266 (*Helix*).
1849 MOUSSON, Land- & Süssw. Moll. Java, p. 23, pl. 2, fig. 6 (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 270 (*Helix*).

Material examined:
    A. Museum Amsterdam.
        Klein Kombuis Id., soil fauna, Jan. 27, 1922, leg. K. W. DAM-
        MERMAN.
        Trouwers Id., Febr. 18, 1932, leg. DARNA and SAÄN.
        Klapper Id., Febr. 18–23, 1932, leg. DARNA and SAÄN.

Previous records in literature:

> Krakatau Id., 1867 MARTENS, l.c.
> Krakatau Id. 1888 TENISON WOODS, Proc. Linn. Soc. N. S. Wales, (2) Vol. 3, p. 1031 (*Helix cryptopila*).
> Krakatau Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (*Trichochloritis*).
> Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6, p. 142 (*Chloritis helicinoides* var. *cryptopila*).
> Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 86 (*Chloritis* sp.).

The species seems to be confined to West Java and the above-mentioned islands off the coast. Klein Kombuis, Trouwers Id. and Klapper Id. are new records. *Chloritis helicinoides* was one of the five species known from Krakatoa before the eruption of 1883, but it was never recorded there again after that catastrophe.

Chloritis transversalis (Mousson)

1857 MOUSSON, Journ. de Conch. Vol. 6, p. 158, pl. 6, fig. 5 (*Helix*)

1867 MARTENS, Ostas. Landschn. p. 273 (*Helix*).

Previous records in literature:

> Madoera Id., 1857 MOUSSON, l.c.
> Madoera Id., 1859 ZOLLINGER, Natuurk. Tijdschr. Ned. Indië, Vol. 18, p. 424 (*Helix transovalis*).
> Madoera Id., 1867 MARTENS, l.c.
> Madoera Id., 1903 GUDE, Journ. Malac. Vol. 10, p. 56 (*Eulota*).
> Madoera Id., 1914 LESCHKE, Mitt. naturhist. Mus. Hamburg, Vol. 31, p. 235 (*Eulota*).
> Madoera Id., 1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 85.
> Madoera Id., 1933 RENSCH, Sitz. Ber. Ges. naturf. Freunde Berlin, p. 506.

The species is known from Madoera and Bali. In Soemba lives a special race: *Chl. transversalis conjecta* Smith (RENSCH, ll.cc.).

Amphidromus contrarius subsp. *baweanicus* Fruhstorfer

1905 FRUHSTORFER, Nachr. Blatt, Vol. 37, p. 198–199.

Material examined:
> A. Museum Amsterdam.
> > Bawean Id.
> > Bawean Id., leg. P. A. OUWENS.
> > Bawean Id., South part, May 1928, leg K. W. DAMMERMAN.
> C. Museum Leiden.
> > Bawean Id., leg. H. FRUHSTORFER.
> > Bawean Id., leg. E. F. JOCHIM.

Previous records in literature:
>
> Bawean Id., 1905 Fruhstorfer, l.c.
>
> Bawean Id., 1932 Rensch, Zool. Jahrb. (Syst.) Vol. 63, p. 96.
>
> Bawean Id., 1936 Rensch, Gesch. Sunda Bogens, p. 110.

The subspecies is only known from Bawean. It is a local form of a "Rassenkreis" occurring in East Java and several of the Lesser Sunda Islands.

Amphidromus filozonatus (Martens)

1867 Martens, Ostas. Landschn. p. 358–359, pl. 21, fig. 4 (*Bulimus*).

1900 Pilsbry, Man. of Conch. (2) Vol. 13, p. 202, pl. 64, fig. 4.

Material examined:
>
> A. Museum Amsterdam.
>> Madoera Id., Soemenep.
>
> B. Museum Buitenzorg.
>> Madoera Id., Pamekasan, leg. P. A. Ouwens.
>
> C. Museum Leiden.
>> Madoera Id., leg. E. F. Jochim.
>> Bawean Id., leg. H. Rolle.

Previous records in literature:
>
> Madoera Id., 1867 Martens, l.c.
>
> Madoera Id., 1903 Gude, Journ. Malac. Vol. 10, p. 56.
>
> Madoera Id., 1914 Leschke, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 231.
>
> Madoera Id., 1932 Rensch, Zool. Jahrb. (Syst.) Vol. 63, p. 96 (*Amphidromus contrarius filozonatus*).

The species was known from East Java and Madoera. Bawean is a new record.

Amphidromus heerianus var. *poecilus* Pilsbry

1900 Pilsbry, Man. of Conch. (2) Vol. 13, p. 138–139, pl. 46, fig. 19, 20.

Material examined:
>
> A. Museum Amsterdam.
>> Meeuwen Id., March 30, 1932, leg. P. F. Franck.
>> Meeuwen Id., Aug. 6, 1932, leg. P. F. Franck.

Both the species and the variety were known from Java only. Meeuwen Id. is a new locality.

Amphidromus inversus (Müller)

1774 Müller, Hist. Verm., Vol. 2, p. 93 (*Helix*).

1867 Martens, Ostas. Landschn. p. 337–338 (*Bulimus*).

1900 PILSBRY, Man. of Conch. (2) Vol. 13, p. 167, pl. 56, fig. 91, 92.

Material examined:
    A. Museum Amsterdam.
          Klein Kombuis Id., Nov. 11, 1920, leg. K. W. DAMMERMAN.
          Klein Kombuis Id., May 1923, leg. W. M. DOCTERS VAN
            LEEUWEN.
    C. Museum Leiden.
          Poeloe Klappa, Duizend Ids., leg. J. C. VAN DER MEER MOHR.
Previous records in literature:
          Krakatau Id.,
          Dwars in den Weg Id., } 1867 MARTENS, lc.
          Krakatau Id.,   1903 GUDE, Journ. Malac. Vol. 10,
          Dwars in den Weg Id., }   p. 56.
          Krakatau Id., 1925 VAN BENTHEM JUTTING, Treubiá, Vol. 6,
            p. 142.
          Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 86.
          Duizend Ids., 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67,
            p. 173.

The records for Krakatau refer to the occurrence of *Amphidromus inversus* before the eruption of 1883. After that catastrophe the species is no longer found there.

The distribution of this species comprises the Malay Peninsula, Borneo, Sumatra and the above-mentioned islands off the coast of Java. Klein Kombuis is a new locality and Poeloe Klappa is a more precise location in the Duizend Ids. Archipelago. The reference to Java proper needs further confirmation.

Amphidromus palaceus (Mousson)

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 28, 108, pl. 3, fig. 1 (*Bulimus*).

1867 MARTENS, Ostas. Landschn. p. 352 (*Bulimus*).

1900 PILSBRY, Man. of Conch. (2) Vol. 13, p. 134, pl. 47, fig. 1, 2, 4, 5, 6.

Material examined:
    A. Museum Amsterdam.
          Madoera Id., Pamekasan, leg. P. A. OUWENS.
          Madoera Id., Djoemiang, leg. P. A. OUWENS.
          Noesa Kembangan, Nature Reserve Gligir, Febr. 15-16, 1927,
            leg. P. F. FRANCK.
    C. Museum Leiden.
          Madoera Id., leg. E. F. JOCHIM (var. *pura* Mouss.).
Previous records in literature:
          Noesa Kembangan, 1900 MARTENS, Nachr. Blatt, Vol. 32 p. 17.

Noesa Kembangan, 1900 Pilsbry, l.c.
Noesa Kembangan,) 1935 Paravicini, Arch. Moll. Kunde, Vol.
Madoera Id.,   (67, p. 173.

The species is known to occur in Sumatra, Java, Madoera and Noesa Kembangan.

Amphidromus perversus (Linné)

1758 Linné, Syst. Nat. Ed. X, p. 772 (Helix).
1774 Müller, Hist. Verm., Vol. 2, p. 94 (Helix interrupta).
1867 Martens, Ostas. Landschn. p. 344–347, pl. 20, fig. 1, 2, 3, 5, 6, 8, 9 (Bulimus interruptus), p. 349–351, pl. 20, fig. 13 (Bulimus perversus).
1900 Pilsbry, Man. of Conch. (2) Vol. 13, p. 147, pl. 51, fig. 47–52 (perversus), p. 150, pl. 52, fig. 53–56 (interruptus).

Material examined:
  A. Museum Amsterdam.
        Karimon Djawa Ids., Oct. 1920, leg. H. C. Delsman.
        Karimon Djawa Ids., May 1926, leg. K. W. Dammerman.
        Karimon Djawa Ids., Poeloe Kemoedjan, May 16, 1926, leg. P. F. Franck.
        Karimon Djawa Ids., wood near the churchyard, Nov. 23, 1930, leg. M. A. Lieftinck.
        Karimon Djawa Ids., River Pantjoeran, Bronbeek, Nov. 26, 1930, leg. M. A. Lieftinck.
        Madoera Id., leg. P. A. Ouwens.
  B. Museum Buitenzorg.
        Madoera Id., Djoemiang, leg. P. A. Ouwens.
        Madoera Id., Pamekasan, leg. P. A. Ouwens.
  C. Museum Leiden.
        Madoera Id., Bengkalen, Aug. 1, 1911, leg. P. Buitendijk.
        Madoera Id., 1913, leg. P. Buitendijk.
        Madoera Id., leg. E. F. Jochim.
        Bawean Id., leg. H. Fruhstorfer.
Previous records in literature:
        Noesa Baron, 1849 Mousson, Land- & Süssw. Moll. Java, p. 32 (Bulimus interruptus var. sultanus).
        Noesa Baron, 1867 Martens, Ostas. Landschn. p. 345 (Bulimus interruptus).
        Noesa Baron, 1900 Pilsbry, l.c. p. 150 (interruptus).
        Noesa Baron, 1914 Leschke, Mitt. naturh. Mus. Hamburg, Vol. 31, p. 232 (interruptus).
        Prinsen Id., 1900 Pilsbry, Man of Conch. (2) Vol. 13, p. 161 (aureus).
        Prinsen Id., 1903 Gude, Journ. Malac. Vol. 10, p. 56 (aureus).
        Bawean Id., 1903 Gude, l.c. p. 56 (interruptus).

Bawean Id., ⎫ 1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63,
Madoera Id., ⎭ p. 101.
Bawean Id., 1936 RENSCH, Gesch. Sunda Bogens, p. 110.
Madoera Id., ⎫ 1935 PARAVICINI, Arch. Moll. Kunde, Vol. 67, p.
Kangean Id., ⎭ 173 (*interruptus* and *perversus*).

Both the samples from the Karimon Djawa Ids., collected in
May 1926, consist of shells with very vivid and variegated
coloration.

The species has a wide distribution in the Malay Archipelago.
For detailed information on localities (including Java) I can
refer to RENSCH (Zool. Jahrb. (Syst.) Vol. 63, 1932, p. 101), to
which Noesa Baron, Prinsen Id., and Kangean can be added.
The Karimon Djawa Ids. are a new, and so far unpublished,
record.

Amphidromus perversus fa. *infrapictus* (Martens)

1867 MARTENS, Ostas. Landschn. p. 344 (*Bulimus interruptus* var.
infrapictus).
1900 PILSBRY, Man. of Conch. (2) Vol. 13, p. 153 (*interruptus* fa.
infrapictus).

Previous records in literature:
Bawean Id., 1900 MARTENS, Nachr. Blatt, Vol. 32, p. 17 (*inter-
ruptus* var. *infrapictus*).
Bawean Id., 1900 PILSBRY, l.c.

This form is known from Java, Bali, Bawean and Celebes.

Amphidromus perversus fa. *infraviridis* (Martens)

1867 MARTENS, Ostas. Landschn. p. 344 (*Bulimus interuptus* var.
infraviridis).
1900 PILSBRY, Man. of Conch. (2) Vol. 13, p. 151, pl. 52,
fig. 59–65, pl. 50, fig. 51, 52 (*interruptus* fa. *infraviridis*).

Material examined:
A. Museum Amsterdam.
Kangean Ids., Siboga Expedition.
Kangean Ids., Tambajangan, April 1919, leg. C. BACKER.
Previous records in literature:
Kangean Ids., 1913 SCHEPMAN, Siboga Exp. Monogr. 49–1–f,
p. 457–458 (*interruptus* var. *infraviridis*).

The form is recorded from Java, Kangean and Celebes.

Amphidromus perversus rufocinctus Fruhstorfer

1905 FRUHSTORFER, Nachr. Blatt, Vol. 37, p. 199–200.

Material examined:
- A. Museum Amsterdam.
    - Bawean Id., leg. J. VAN BAREN.
    - Bawean Id., leg. P. A. OUWENS.
    - Bawean Id., North part, June 2, 1920, leg. K. W. DAMMERMAN.
    - Bawean Id., South part, May 1928, leg. K. W. DAMMERMAN.
    - Bawean Id., limestone hill East of Sangkapoera, on stem of Sterculia foetida, Nov. 23, 1937, leg. J. H. COERT.
    - Bawean Id., Telaga Kastobo, 250 m alt., rather common on phonolite and phonolite tufa, Nov. 25–27, 1937, leg. J. H. COERT.

Previous records in literature:
- Bawean Id., 1905 FRUHSTORFER, l.c.
- Bawean Id. (or Karimon Djawa Ids.) 1923 OOSTINGH, Meded. Landb. Hoogesch. p. 153.

Among the 5 shells of *Amphidromus perversus rufocinctus* from Bawean Id., collected May 1928, there is one very elongated specimen, measuring: height 52.5, breadth 23.5, height aperture 23 mm.

OOSTINGH (l.c.) was not certain where the specimens, collected by VAN BAREN, came from. I feel sure that this must have been Bawean, and *not* the Karimon Djawa Islands.

Amphidromus perversus rufocinctus fa. *sankapurus* Fruhstorfer

1905 FRUHSTORFER, Nachr. Blatt, Vol. 37, p. 200.

Material examined:
- A. Museum Amsterdam.
    - Bawean Id., leg. H. FRUHSTORFER.

Previous records in literature:
- Bawean Id., 1905 FRUHSTORFER, l.c.

Only known from Bawean Id.

Amphidromus perversus fa. *strigosus* (Martens)

1867 MARTENS, Ostas. Landschn. p. 344, pl. 20, fig. 3, 8 (*Bulimus interruptus* var. *strigosus*).

1900 PILSBRY, Man. of Conch. (2) Vol. 13, p. 151, pl. 52, fig. 57, 58 (*interruptus* fa. *strigosus*).

Material examined:
- C. Museum Leiden.
    - Madoera Id., leg. E. F. JOCHIM.

Previous records in literature:
- Noesa Baron, 1867 MARTENS, l.c.

The form is known from Java, Noesa Baron, Bali, Borneo and Celebes. The island of Madoera is a new habitat.

Amphidromus porcellanus (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 266 (*Bulimus*).

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 33–34, pl. 3, fig. 4 (*Bulimus*).

1867 MARTENS, Ostas. Landschn. p. 365–366 (*Bulimus*).

Material examined:
A. Museum Amsterdam.
   Krakatau Id., 500–800 m alt., Jan. 1922, leg. W. M. DOCTERS VAN LEEUWEN.
   Sebesi Id., North part, April 1921, leg. K. W. DAMMERMAN.
B. Museum Buitenzorg.
   Krakatau Id., April 25, 1919, leg. A. L. J. SUÑIER.
   Krakatau Id., 500–800 m alt., Jan. 1922, leg. W. M. DOCTERS VAN LEEUWEN.
   Krakatau Id., April 1933, leg. K. W. DAMMERMAN.
   Sebesi Id., North part, April 1921, leg. K. W. DAMMERMAN.
Previous records in literature:
   Krakatau Id., 1909 JACOBSON, Jaarversl. Topogr. Dienst, Vol. 14 (1908) list opposite p. 206 (*Bulimulus (Amphidromus) porcellanus*).
   Krakatau Id., } 1925 VAN BENTHEM JUTTING, Treubia, Vol. 6,
   Sebesi Id.,   }   p. 143.
   Krakatau Id., 1929 DAMMERMAN, Krakatau's New Fauna, p. 86, 114.
   Krakatau Id., } 1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 96
   Sebesi Id.,   }   (*contrarius porcellanus*).
   Krakatau Id., } 1934 RENSCH, Trop. Binnengew. Vol. 4, p. 755
   Sebesi Id.,   }   (*contrarius porcellanus*).

The species is recorded from South Sumatra, Krakatau, Sebesi and West Java.

Pseudopartula galericulum (Mousson)

1849 MOUSSON, Mitth. naturf. Ges. Zürich, Vol. 1, Part 3, p. 266 (*Bulimus*).

1849 MOUSSON, Land- & Süssw. Moll. Java, p. 34, pl. 3, fig. 5 (*Bulimus*).

1867 MARTENS, Ostas. Landschn. p. 324 (*Helix*).

1930 RENSCH, Zool. Anz. Vol. 92, p. 183–187, fig. 2.

Material examined:
A. Museum Amsterdam.
   Noesa Kembangan, June 1931, leg. Mrs. A. C. VAN HEURN.
   Noesa Kembangan, March 1932, leg. W. M. DOCTERS VAN LEEUWEN.

B. Museum Buitenzorg.
     Madoera Id., Pamekasan, leg. P. A. OUWENS.
Previous records in literature:
     Noesa Kembangan, 1912 SCHEPMAN, Proc. malac. Soc. London,
          Vol. 10, p. 234.

The species is only known from West Java, Noesa Kembangan
and Madoera.

Bradybaena similaris (Férussac)

1821 FÉRUSSAC, Hist. Nat. génér. & part. Moll. Tabl. Limaçons,
     p. 47 (*Helix*).
1831 FÉRUSSAC, in: RANG, Ann. Sci. nat. Vol. 24, 93, p. 15
     (*Helix*).
1867 MARTENS, Ostas. Landschn. p. 270–271 (*Helix*).

Material examined:
     A. Museum Amsterdam.
          Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
          Noordwachter Id., Sept. 1921, leg. H. BOSCHMA. .
          Klein Kombuis Id., May 1923, leg. W. M. DOCTERS VAN LEEUWEN.
Previous records in literature:
          Noesa Kembangan, 1935 PARAVICINI, Arch. Moll. Kunde, Vol.
          67, p. 173.

Among the sample from Noordwachter Id., collected by H.
BOSCHMA, Sept. 1921, there are two shells which show a brown
peripheral band.
The species is a tropical cosmopolite. In the Malay Archi-
pelago it was so far known from Sumatra, Java, Noesa Kem-
bangan, Timor and the Kei Ids. Noordwachter and Klein
Kombuis are new records.

Gulella (*Indoennea*) *bicolor* (Hutton)

1834 HUTTON, Journ. As. Soc. Bengal, Vol. 3, p. 86 (*Pupa*).
1867 MARTENS, Ostas. Landschn. p. 384–385 (*Pupa*).
1932 RENSCH, Zool. Jahrb. (Syst.) Vol. 63, p. 5, fig. 1.

Material examined:
     A. Museum Amsterdam.
          Noordwachter Id., Sept. 8, 1921, leg. K. W. DAMMERMAN.
          Edam Id., March 19, 1920, leg. K. W. DAMMERMAN.
Previous records in literature:
          Madoera Id., 1867 MARTENS, l.c.
          Madoera Id., 1914 LESCHKE, Mitt. naturh. Mus. Hamburg, Vol.
          31, p. 223.
          Madoera Id., 1932 RENSCH, l.c.

It is a tropical cosmopolite. In the Malay Archipelago it is found in most of the islands where serious collecting has been done. Noordwachter and Edam are new localities.

Polymesoda eximia (Dunker)

1852 DUNKER, Zeitschr. Malak. Vol. 9, p. 51 (*Cyrena*).
1897 MARTENS, in: WEBER, Erg. Reise Nied. Ost Indien, Vol. 4, p. 98 (*Cyrena*).
Previous records in literature:
        Noesa Kembangan, 1897 MARTENS, l.c.
        Noesa Kembangan, 1914 LESCHKE, Mitt. naturh. Mus. Hamburg
           Vol. 31, p. 268 (*Cyrena*).

The species is known from Java and Noesa Kembangan. According to PRASHAD (Siboga Exp. Monogr. 53-*c*, 1932, p. 176) it is probable that *Polymesoda eximia* ought to be included into *P. bengalensis* (Lam.). If that would prove to be true, the area, occupied by the species in the Indo-Pacific region, would increase enormously.

Corbicula ducalis Prime

1862 PRIME, Proc. Boston Soc. Nat. Hist. Vol. 7, p. 274.
1930 PRASHAD, Mem. Ind. Mus. Vol. 9, p. 195–196, pl. 24, fig. 7–12.
Material examined:
    A. Museum Amsterdam.
        Dapoer Id., 1938, leg. J. D. F. HARDENBERG.
        Alkmaar Id., 1938, leg. J. D. F. HARDENBERG.
        Hoorn Id., 1938, leg. J. D. F. HARDENBERG.
        Haarlem Id., 1938, leg. J. D. F. HARDENBERG.
        Schiedam Id., 1938, leg. J. D. F. HARDENBERG.
        Purmerend Id., 1938, leg. J. D. F. HARDENBERG.
        Amsterdam Id., 1938, leg. J. D. F. HARDENBERG.
        Middelburg Id., 1938, leg. J. D. F. HARDENBERG.
        Enkhuizen Id., 1938, leg. J. D. F. HARDENBERG.
        Edam Id., 1938, leg. J. D. F. HARDENBERG.
Previous records in literature:
        Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 63, p. 231.

The shells from the islands in the Bay of Batavia, although fresh in most of the cases, must be transported from the main island, as there is hardly any freshwater in those minuscule islands. The same holds good for the single valve from Lang Id. (fide OOSTINGH, l.c.).

Corbicula ducalis is widely distributed in Sumatra, Java, and Lombok. According to PRASHAD the occurrence in Borneo and Celebes needs further confirmation.

Corbicula pullata (Philippi)

1850 PHILIPPI, Abb. Conch. Vol. 3, p. 10 (*Cyrena*).
1930 PRASHAD, Mem. Ind. Mus. Vol. 9, p. 201–202, pl. 26, fig. 1–6.

Previous records in literature:
Lang Id., 1931 OOSTINGH, Arch. Moll. Kunde, Vol. 67, p. 232.

The record for Lang Id. is as problematic as that of *Corbicula ducalis* Prime in the same locality (fide OOSTINGH, l.c.).

The species is known besides from Borneo, Sumatra, Java and Soembawa.

IV. ARRANGEMENT OF SPECIES ACCORDING TO THE ISLANDS

1. MEEUWEN ISLAND (Poeloe Peutjang)

Leptopoma vitreum (Lesson).
Hemiplecta bataviana (Von dem Busch).
Hemiplecta humphreysiana (Lea).
Amphidromus heerianus var. *poecilus* Pilsbry.

2. PRINSEN ISLAND (Poeloe Panaitan)

Pythia pantherina (Adams).
Opeas gracile (Hutton).
Amphidromus perversus (Linné).

3. POPOLE ISLAND

Landouria rotatoria (Von dem Busch).

4. KRAKATAU ISLAND (Poeloe Rakata) [1]

Theodoxis bicolor (Récluz).
*Cyclophorus perdix (Broderip & Sowerby).
Pythia chrysostoma Tapparone Canefri.
Succinea javanica Schepman.
Gastrocopta pediculus ovatula (Möllendorff).
Subulina octona (Bruguière).
Opeas gracile (Hutton).
Kaliella doliolum (Pfeiffer).
Kaliella indifferens Boettger.

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[1] The species, marked with an *, refer to records before the eruption of Krakatau in 1883. After this catastrophe the species were no longer found there. All other species are modern records.

Hemiplecta bataviana (Von dem Busch).
Hemiplecta javacensis (Férussac).
Durgellina convexoconica (Möllendorff).
Chloritis helicinoides (Mousson).
Amphidromus inversus (Müller).
Amphidromus porcellanus (Mousson).

5. VERLATEN ISLAND (Poeloe Sertoeng)

Theodoxis oualaniensis var. *nigrobifasciata* (Martens).
Stenothyra ventricosa (Quoy & Gaimard).
Thiara tuberculata (Müller).
Pythia chrysostoma Tapparone Canefri.
Pythia pantherina (Adams).
Gastrocopta pediculus ovatula (Möllendorff).
Opeas gracile (Hutton).
Kaliella indifferens Boettger.

6. LANG ISLAND (Poeloe Rakata ketjil)

Melampus fasciatus (Deshayes).
Melampus luteus (Quoy & Gaimard).
Pythia plicata (Férussac).
Pythia scarabaeus (Linné).
Corbicula ducalis Prime.
Corbicula pullata (Philippi).

7. SEBESI ISLAND

Leptopoma vitreum (Lesson).
Pupina superba Pfeiffer.
Thiara tuberculata (Müller).
Pythia pantherina (Adams).
Pythia scarabaeus (Linné).
Vaginula bleekeri (Keferstein).
Phaedusa sumatrana (Martens).
Prosopeas achatinaceum (Pfeiffer).
Prosopeas turriculum (Martens).
Kaliella indifferens Boettger.
Durgellina convexoconica (Möllendorff).
Helicarion lineolatus Martens.
Microparmarion strubelli Simroth.
Landouria rotatoria (Von dem Busch).
Amphidromus porcellanus (Mousson).

8. DWARS IN DEN WEG ISLAND (Poeloe Soengian)

Trochomorphá bicolor Martens.
Hemiplecta virens (Martens).
Dyakia martini (Pfeiffer).
Amphidromus inversus (Müller).

9. DUIZEND ISLANDS

Neritina pulligera (Linné).
Amphidromus inversus (Müller).

10. NOORDWACHTER ISLAND

Hydrocena javana (Möllendorff).
Pythia pantherina (Adams).
Semperula maculata (Templeton).
Succinea gracilis Lea.
Tornatellina subcylindrica Quadras & Möllendorff.
Gastrocopta euryomphala Pilsbry.
Gastrocopta pediculus ovatula (Möllendorff).
Subulina octona (Bruguière).
Opeas gracile (Hutton).
Stenopylis coarctata (Möllendorff).
Kaliella doliolum (Pfeiffer).
Bradybaena similaris (Férussac).
Gulella bicolor (Hutton).

11. POELOE KLAPPA (DUIZEND IDS.)

Amphidromus inversus (Müller).

12. ZUIDWACHTER ISLAND (Poeloe Paniki)

Tornatellina subcylindrica Quadras & Möllendorff.
Gastrocopta pediculus ovatula (Möllendorff).
Opeas gracile (Hutton).
Stenopylis coarctata (Möllendorff).
Kaliella doliolum (Pfeiffer).

13. KLEIN KOMBUIS ISLAND (Poeloe Bokor)

Omphalotropis columellaris Quadras & Möllendorff.
Pythia pantherina (Adams).
Gastrocopta euryomphala Pilsbry.
Gastrocopta lyonsiana (Ancey).
Gastrocopta pediculus ovatula (Möllendorff).

Opeas gracile (Hutton).
Stenopylis coarctata (Möllendorff).
Kaliella doliolum (Pfeiffer).
Chloritis helicinoides (Mousson).
Amphidromus inversus (Müller).
Bradybaena similaris (Férussac).

14. DAPOER ISLAND

Corbicula ducalis Prime.

15. MIDDELBURG ISLAND (Poeloe Ramboet)

Septaria suborbicularis (Sowerby).
Melampus fasciatus (Deshayes).
Ellobium auris-judae (Linné).
Corbicula ducalis Prime.

16. AMSTERDAM ISLAND (Poeloe Oentoeng Djawa)

Septaria suborbicularis (Sowerby).
Assiminea brevicula var. *miniata* (Martens).
Thiara scabra (Müller).
Thiara tuberculata (Müller).
Corbicula ducalis Prime.

17. SCHIEDAM ISLAND (Poeloe Oebi Ketjil or Poeloe Gosong)

Corbicula ducalis Prime.

18. ROTTERDAM ISLAND (Poeloe Oebi)

Melampus fasciatus (Deshayes).

19. KERKHOF ISLAND (Poeloe Kelor)

Pila conica (Gray).
Assiminea brevicula (Pfeiffer).
Allochroa conica (Pease).
Melampus fasciatus (Deshayes).
Cassidula auris-felis (Bruguière).
Cassidula labio Möllendorff.

20. PURMEREND ISLAND (Poeloe Sakit)

Neritina violacea (Gmelin).
Pila conica (Gray).

Melampus fasciatus (Deshayes).
Cassidula auris-felis (Bruguière).
Cassidula mustelina (Deshayes).
Corbicula ducalis Prime.

21. HAARLEM ISLAND (Poeloe Noesi or Poeloe Ajer ketjil)

Corbicula ducalis Prime.

22. HOORN ISLAND (Poeloe Ajer)

Pila conica (Gray).
Semperula maculata (Templeton).
Corbicula ducalis Prime.

23. EDAM ISLAND (Poeloe Damar Besar)

Neritina violacea (Gmelin).
Pila conica (Gray).
Tornatellina cylindrica Sykes.
Gastrocopta euryomphala Pilsbry.
Gastrocopta lyonsiana (Ancey).
Gastrocopta pediculus ovatula (Möllendorff).
Opeas gracile (Hutton).
Kaliella doliolum (Pfeiffer).
Gulella bicolor (Hutton).
Corbicula ducalis Prime.

24. ALKMAAR ISLAND (Poeloe Damar Ketjil)

Melampus fasciatus (Deshayes).
Corbicula ducalis Prime.

25. ENKHUIZEN ISLAND (Poeloe Tala)

Pila conica (Gray).
Stenopylis coarctata (Möllendorff).
Corbicula ducalis Prime.

26. LEIDEN ISLAND (Poeloe Njamoek)

Assiminea borneensis (Issel).

27. SMALL ISLAND NEAR KARANG ANJER

Landouria ciliocincta (Möllendorff).

28. POELOE PANDJANG

Cassidula auris-felis (Bruguière).
Pythia plicata (Férussac).

29. Karimon Djawa Islands

Pila conica (Gray).
Thiara tuberculata (Müller).
Melampus fasciatus (Deshayes).
Melampus variabilis Gassies.
Pythia pantherina (Adams).
Anisus convexiusculus (Hutton).
Opeas gracile (Hutton).
Kaliella doliolum (Pfeiffer).
Microcystina nana (Möllendorff).
Hemiplecta javacensis (Férussac).
Amphidromus perversus (Linné).

30. Bawean Island

Japonia trochulus (Martens).
Viviparus javanicus (Von dem Busch).
Pila conica (Gray).
Brotia testudinaria (Von dem Busch).
Thiara scabra (Müller).
Thiara granifera (Lamarck).
Thiara tuberculata (Müller).
Prosopeas achatinaceum (Pfeiffer).
Hemiplecta bataviana (Von dem Busch).
Hemiplecta bataviana var. *duplocincta* (Möllendorff).
Amphidromus contrarius subsp. *baweanicus* Fruhstorfer.
Amphidromus filozonatus (Martens).
Amphidromus perversus (Linné).
Amphidromus perversus fa. *infrapictus* (Martens).
Amphidromus perversus rufocinctus Fruhstorfer.
Amphidromus perversus rufocinctus fa. *sankapurus* Fruhstorfer.

31. Madoera Island

Theodoxis angulosus (Récluz).
Theodoxis bicolor (Récluz).
Theodoxis corona (Linné).
Theodoxis flavovirens (Von dem Busch).
Theodoxis oualaniensis (Lesson).
Theodoxis oualaniensis var. *diremta* (Martens).
Neritodryas cornea (Linné).
Neritina violacea (Gmelin).
Neritina turrita (Gmelin).

Neritina variegata Lesson.
Neritina pulligera (Linné).
Neritina squamipicta (Récluz).
Septaria cumingiana (Récluz).
Septaria suborbicularis (Sowerby).
Septaria tessellata (Lamarck).
Cyclophorus perdix (Brod. & Sow.).
Cyclophorus rafflesi (Brod. & Sow.).
Pupina treubi Boettger.
Viviparus javanicus (Von dem Busch).
Pila conica (Gray).
Bithynia truncata (Eyd. & Soul.).
Brotia testudinaria (Von dem Busch).
Thiara scabra (Müller).
Thiara granifera (Lamarck).
Thiara crenulata (Deshayes)
Thiara obesula (Brot).
Thiara plicaria (Born).
Thiara punctata (Lamarck).
Thiara tuberculata (Müller).
Laemodonta imperforata (Adams).
Melampus fasciatus (Deshayes).
Melampus luteus (Quoy & Gaimard).
Cassidula auris-felis (Bruguière).
Cassidula mustelina (Deshayes).
Pythia castanea (Lesson).
Pythia plicata (Férussac).
Pythia scarabaeus (Linné).
Pythia undata (Lesson).
Ellobium auris-judae (Linné).
Ellobium auris-midae (Linné).
Ellobium subnodosum (Metcalfe).
Lymnaea javanica (Mousson).
Anisus convexiusculus (Hutton).
Succinea listeri Smith.
Succinea obesa Martens.
Ena glandula (Mousson).
Hemiphaedusa cornea (Philippi).
Phaedusa corticina (Von dem Busch).
Phaedusa heldi (Küster).
Opeas gracile (Hutton).

Eurybasis conus (Philippi).
Eurybasis multicarinata (Boettger).
Hemiplecta bataviana (Von dem Busch).
Hemiplecta javacensis (Férussac).
Dyakia rumphii (Von dem Busch).
Landouria rotatoria (Von dem Busch).
Landouria squamulosa (Martens).
Chloritis transversalis (Mousson).
Amphidromus filozonatus (Martens).
Amphidromus palaceus (Mousson).
Amphidromus perversus (Linné).
Amphidromus perversus fa. *strigosus* (Martens).
Pseudopartula galericulum (Mousson).
Gulella bicolor (Hutton).

32. KANGEAN ISLANDS

Geophorus rollei (Sykes).
Cyclotus kangeanus Schepman.
Ellobium auris-midae (Linné).
Hemiplecta javacensis (Férussac).
Hemiplecta kangeänensis Schepman.
Landouria kangeanensis (Schepman).
Landouria rotatoria (Von dem Busch).
Amphidromus perversus (Linné).
Amphidromus perversus fa. *infraviridis* (Martens).

33. NOESA BARON

Melampus luteus (Quoy & Gaimard).
Pythia pantherina (Adams).
Phaedusa heldi var. *baronensis* (Mousson).
Phaedusa moritzi (Mousson).
Eurybasis conus (Philippi).
Amphidromus perversus (Linné).
Amphidromus perversus fa. *strigosus* (Martens).

34. NOESA KEMBANGAN

Theodoxis corona (Linné).
Neritina violocea (Gmelin).
Neritina turrita (Gmelin).
Neritina variegata Lesson.
Neritina pulligera (Linné).
Septaria suborbicularis (Sowerby).

Leptopoma altum Möllendorff.
Leptopoma vitreum (Lesson).
Japonia trochulus (Martens).
Cyclophorus perdix (Brod. & Sow.).
Cyclophorus rafflesi (Brod. & Sow.).
Cyclotus corniculum (Mousson).
Pila conica (Gray).
Pila polita (Deshayes).
Assiminea sinensis Nevill.
Assiminea subeffusa Boettger.
Brotia testudinaria (Von dem Busch).
Auriculastra subula (Quoy & Gaimard).
Melampus fasciatus (Deshayes).
Cassidula mustelina (Deshayes).
Pythia pantherina (Adams).
Pythia scarabaeus (Linné).
Pythia trigona (Troschel).
Phaedusa javana (Pfeiffer).
Prosopeas achatinaceum (Pfeiffer).
Trochomorpha planorbis (Lesson).
Hemiplecta bataviana (Von dem Busch).
Hemiplecta humphreysiana (Lea).
Helicarion adolfi Boettger.
Landouria ciliocincta (Möllendorff).
Landouria rotatoria (Von dem Busch).
Chloritis crassula (Philippi).
Chloritis fruhstorferi Möllendorff.
Amphidromus palaceus (Mousson).
Pseudopartula galericulum (Mousson).
Bradybaena similaris (Férussac).
Polymesoda eximia (Dunker).

35. TROUWERS ISLAND (Poeloe Tindjil)

Leptopoma vitreum (Lesson).
Chloritis helicinoides (Mousson).

36. KLAPPER ISLAND (Poeloe Deli)

Leptopoma vitreum (Lesson).
Pythia pantherina (Adams).
Landouria winteriana (Pfeiffer).
Chloritis helicinoides (Mousson).

V. ZOOGEOGRAPHICAL REMARKS ESPECIALLY IN RELATION TO JAVA

Before entering into the zoogeographical reflections of this paragraph a few remarks of a more technical nature will have to precede.

As I pointed out in the beginning of this paper I am only too well aware that the combination of islands, proposed here, is an artificial one. The islands do not form a harmonious entity, but are often divergent in history and physiognomy. The only common feature shared by them all is their unanimous dependence on the main island Java. This dependency is not to be understood in such a way that all the islands necessarily once formed part of Java, having become detached at more or less remote dates, but rather that their fauna is either immediately derived from Java, or has at least so many affinities to the fauna of the main island that its origin is not far to seek.

In the second place I must draw attention to a matter of common knowledge which finds due confirmation in the present study, viz. that, when comparing the fauna of a large territory with that of a small site under similar general conditions of latitude, climate, etc., the small locus is apparently always in advantage as regards abundance of species and of specimens at the rate of numbers per meter square.

This incompatibility, however, can be readily accounted for by the fact that restricted areas, with comparatively mild orographical features and light modes of exploration, are much easier of access and far better investigated than large regions presenting as a rule heavier obstructions, both physical and finantial, to the student.

Consequently the relatively higher ratio of faunal elements, i.c. mollusca, yielded by a small spot is caused by the more intense exploration there and not, or not in the first place, by the greater amount of individuals per unit square.

Now in the present case this discrepancy is fortunately almost entirely obviated by the favourable circumstance that in Java malacology has been studied for more than a century already, thus securirg a solid base for analysis and comparison of the Java fauna with the faunules of its satellites.

The islands surrounding Java are without exception continental islands i.e. they are no formations sui generis springing up right from abyssal depths like the oceanic islands, but they were

22

connected in the past with a continent or with a larger island (i.c. Java) and became cut off by subsidence of the land between.

Whereas the fauna of oceanic islands must have entirely immigrated after their emergence from the waves, the fauna of the continental islands is, at least partly, autochthonous and inherited from the main island. Later other elements can have been added by oversea transport.

Therefore the fauna of oceanic islands is nearly always disharmonious. It consists of species which had the good luck to reach it. Other species, less fortunate, are missing.

The fauna of the continental islands, on the contrary, is more harmonious, agreeing in broad outline with the neighbouring continent or island whence it has been derived.

Even we can say that the total amount of species inhabiting a satellite island is a function of its size and of the distance separating it from the main country, the population being directly proportional to the size and inversely proportional to the advanced position of the outlying island. Discrepancies, however, do occur, but we will no longer insist on this matter here.

To the original stock derived from the ancestral country newcomers can have been added. Generally a precise discrimination between these categories cannot be given with certainty, and therefore the separation between an ancient stock and modern accessions is entirely academic.

Although not oceanic in the true sense of the word, yet the islands of the Krakatau group have received quite a new mollusc fauna by import only. From the original fauna no snail survived and the species thriving there at present are introduced by currents and drift material. The influence of other agencies as wind, birds or man, if any, is so little that it can be neglected.

About the rapidity with which such migrations take place only the case of Krakatau gives a certain direction. For the other islands the evidence goes back so far into the geological history that it lies beyond our power of observation nor is it accessible for experimental investigation.

For the zoogeographical remarks which are going to follow hereafter it seems most convenient to divide the islands around Java into three sections: a Western, a Northern and a Southern section.

The Western Section comprises all the islands in Soenda Strait (nos. 1–8). When considering these we have to compare

their fauna with that of their two large neighbours: Java on the one and Sumatra on the opposite side.

There can be no doubt that the zoogeographical affinities of the majority of the Soenda Strait islands: Meeuwen Id., Prinsen Id., Popole, Krakatau, Verlaten Id. and Lang Id. are entirely pointing to Java. With a single exception all the mollusc inhabitants of these islands are also found in Java. This exception is formed by *Gastrocopta pediculus ovatula*, a small species from the surface fauna which, owing to its minute size and its hidden mode of life, has probably thus escaped the notice of collectors in Java, but which is almost surely to be expected here too. The species is not recorded from Sumatra either.

On the other hand species like *Leptopoma vitreum*, *Amphidromus heerianus* var. *poecilus*, *Landouria rotatoria*, *Succinea javanica*, *Kaliella indifferens*, *Chloritis helicinoides*, *Durgellina convexoconica* are not recorded from Sumatra. [1]) Another species of *Kaliella*, *K. doliolum*, is not recorded from Sumatra either, but as it pops up again in Poeloe Berhala and in the Malay Peninsula the supposition that it will be found in Sumatra too does not seem too hazardous.[2])

Only the islands Sebesi and Dwars in den Weg possess a few species which positively demonstrate a somewhat closer connection with Sumatra. Here we find *Pupina superba*, *Phaedusa sumatrana*, and *Helicarion lineolatus* (all Sebesi) and *Hemiplecta virens* and *Dyakia martini* (both Dwars in den Weg) which are not recorded eastwards beyond these two islands. These five species are the most advanced outposts of Sumatra towards Java.

The reversed condition: absence in Sumatra, presence in Java, is represented by *Leptopoma vitreum*, *Durgellina convexoconica*, *Landouria rotatoria* and *Microparmarion strubelli* (all Sebesi).

All other species inhabiting Sebesi and Dwars in den Weg have an uninterrupted distribution throughout Java and Sumatra. Only *Prosopeas turriculum* is not mentioned from Sumatra, but as it turns up again in Siam, it may be possible that its occurrence in Sumatra has been overlooked so far. [2])

For the islands of the Northern Section, off the N-coast of

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[1]) In 1929 DAMMERMAN (On the Zoogeography of Java, Treubia, Vol. 11, p. 20) stated already that not a single *Kaliella* was recorded from Sumatra. This condition is still unchanged.

[2]) *Kaliella doliolum* and *Prosopeas turriculum* demonstrate a faunistic relationship between Java and Asia, while evading Sumatra (cfr. DAMMERMAN, On the Zoogeography of Java, Treubia, Vol. 11, 1929, p. 19 and 21).

Java (Duizend Ids. to Kangean Ids. inclusive) we have to consider whether their faunistic affinities are leading to Java only or whether other sources of origin are also perceptible. For this purpose we will, like in the preceding section, restrict ourselves to the land and freshwater species exclusively, leaving brackish water or "submarine" species entirely out of account.

The chief centre except Java, whence faunal participants can be expected, is the large island on the opposite side of the Java Sea: Borneo. Other sources of migration, perhaps effective in other systematic groups, do not show such influence in the mollusca. The position of the Kangean Islands, being a little different, will be discussed separately at the end of the Northern section.

The true freshwater species – which, on the whole, have a more uniform dispersal in the Malay Archipelago than the land shells – scarcely demonstrate a special derivation either from the South (Java) or from the North (Borneo), as most of the species in the satellite islands are equally common to both these Greater Sunda Islands. Only *Bithynia truncata*, *Brotia testudinaria* and *Anisus convexiusculus* point indubitably to a descent from Java, as they have not been recorded from Borneo.

The land snails seem also to have been derived for the greater part from the fauna of Java. There are, moreover, among the land shells a few totally foreign elements, entirely new to the Dutch Archipelago, or with only a very limited distribution so far. These species (*Omphalotropis columellaris*, *Tornatellina cylindrica*, *T. subcylindrica*, *Gastrocopta euryomphala*, *G. lyonsiana*, *G. pediculus ovatula*, *Stenopylis coarctata*), all of minute size, were discovered by DAMMERMAN's careful exploration of the soil fauna in Krakatau, Verlaten Island, Noordwachter, Zuidwachter, Klein Kombuis and Edam. That we have no records from other satellite islands or from Java or Borneo can be explained by the fact that no such methodical surface fauna investigations have been carried out so far in other islands. If in the future similar research will be taken to hand in other stations we may be sure to meet the above-mentioned species – and probably others besides!

Now, however interesting their statement in these satellite islands may be from a faunistic point of view, for a comparison of the faunules of the small islands with the fauna of Java they are entirely inappropriate, because we have not the slightest

evidence, positive or negative, of their occurrence in Java.

Better objects for comparison are the larger genera: *Cyclotus, Cyclophorus, Hemiplecta, Dyakia, Landouria, Bradybaena, Chloritis, Amphidromus*. Their size, colour and unhidden mode of life make them an easy prey for the collector. Therefore most of the travellers, even though not specially trained for snailing, succeeded in securing some of these shells. So by this state of affairs we can dispose of rather satisfactory material, both from the satellite islands and from Java.

Among the landsnails there are three species – of rather small size it is true – which can boast of an unparalleled wide area of distribution, not only in the Malay Archipelago, but also beyond it in the tropics of the Old and New World. Therefore these three: *Opeas gracile, Subulina octona* and *Bradybaena similaris* are of no use for presumptions on areas of distribution or for directions of migration.

There is one species, *Landouria squamulosa*, which is only recorded from Madoera. As I pointed out in the systematic part, it is a rather problematic species which has never been found again. Therefore I cannot propound an opinion as to its origin or affinities.

After substracting the 11 species just-mentioned from the total number of terrestrial species (varieties not included) inhabiting the islands of the Northern Section, we arrive at a total of 31 species to be tested on their Javanese of Bornean affinities.

Among them there are only 7 (*Cyclophorus perdix, Semperula maculata, Prosopeas achatinaceum, Kaliella doliolum, Hemiplecta bataviana, Amphidromus inversus* and *A. perversus*) which are inhabiting Borneo as well as Java. Consequently they do not offer an indication for inferring the source whence the land snails took possession of these islands.

All other species are derived from the Java fauna, a contention, which is not exceptionally hazardous, on the contrary is even perfectly conceivable, if we take into account the closer proximity of the Java coast as compared with the distance to Borneo. Even for the most advanced out-posts: the Karimon Djawa Islands and Bawean, there is no doubt as to the origin of their fauna.

A recent analysis of the fauna of Bawean by RENSCH (l.c., 1916, p. 108–110) based on birds, butterflies and landsnails, fully confirms the fact that the fauna of this island has far greater affinities towards Java than towards Borneo.

Having seen now that the long-distance dispersal of species in N-S direction is of little importance for our problem, the following lines will show as well that there is equally little evidence for an active inter-island exchange of species in the W-E direction.

For a search of this kind we have to consider the very same 31 species, discussed a few lines before. Of these we see that 24 are recorded from 1 island only, 5 species from 2 islands, 1 species from 3 islands, none from 4 islands and 1 from 5 islands.

We thus obtain in my opinion a conclusive proof that almost no mutual intercourse between the islands existed. And it strengthens the evidence that, for the allocation of their mollusc fauna, all the satellites one by one, separately, are dependent on the main island Java, the mighty backbone with which several of the satellites were connected in a more or less remote time.

The allied ancestry of the islands thus being ascertained, it is equally evident from the list of species in paragraph IV that there are some differences, not in total amount of species, but in their nature, between the islands North of West Java and those off the North-coast of East Java.

More than in any previous part of my account severe criticism is indispensable here, as not each species present in the one and absent in the other region is lending sufficient conclusive support for this thesis. Hence we cannot design by one stroke as "Western" the six species which are only found in the islands North of West Java, or as "Eastern" a score of species recorded from the islands North of East Java and not in the other region. Careful analysis leads to the conclusion that only *Chloritis helicinoides* (Klein Kombuis) and *Amphidromus inversus* (Duizend Islands, Poeloe Klappa, Klein Kombuis) are real "Western" elements, whereas *Chloritis transversalis* (Madoera), *Amphidromus contrarius baweanicus* (Bawean) and *A. filozonatus* (Bawean, Madoera) represent the other category.

The common descent of most of the North-coast islands and the derivation of their mollusca from Java (and from Borneo, if any) dates back as far as the Glacial period for neither in the Karimon Djawa Islands nor in Bawean which are both of prequarternary, here and there even pretertiary origin, elements of an older non-marine mollusc population are to be traced. During the Ice Age when large masses of ice and snow were

lying piled up in the polar regions, so much water was withdrawn from the oceans that the sea-level in the Malay Archipelago was lowered for about 50–60 m. In this way the Java Sea, which is on the average 50 m deep, became a continuous land surface, studded with numerous lakes and river-courses, extending between Borneo and Java.

This is in short the quintessence of an extremely far-reaching and many-sided problem which interests equally geologists as well as biologists and oceanographers and which, in broad outline, has found general acceptance by all of them. [1])

By the above described subsidence of the Java Sea level the fauna of the main island could swarm out to the North, while Bornean species could penetrate southward. It is a peculiar fact that, whereas so many Javanese species experienced no difficulty in migrating northward, not a single specifically Bornean element travelled down southward. [2]) This point of the discussion is still an unsolved problem, for it cannot be the difference in distance only which counts solely. The only result is that a small number of species succeeded to become equally common to Borneo, Java and the intermediate islands, without providing, however, sufficient testimony as to where their cradle lies.

When the Ice Age declined, the climate became more genial, the sea-level rose and the Java Sea reclaimed more and more its ancient territory. Accordingly the lower portions of the peneplain between Borneo and Java became inundated, only

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[1]) G. A. F. MOLENGRAAFF & M. WEBER, Het verband tusschen den plistocenen ijstijd en het ontstaan der Soenda-zee. Versl. Kon. Akad. Wet. Amsterdam, Vol. 28, 1919, p. 497–544.

G. A. F. MOLENGRAAFF, Geologie, in: De Zeeën van Nederlandsch Oost-Indië. Leiden, 1922, p. 272–357.

L. M. R. RUTTEN, Geologische Geschiedenis der Java-Zee, in: Voordrachten over de Geologie van Nederlandsch Oost-Indië. Groningen, 1927, p. 182–191.

J. H. F. UMBGROVE, De Koraalriffen der Duizend-Eilanden (Java-zee). Wetensch. Meded. No. 12, Dienst van den Mijnbouw, 1929, p. 1–47.

[2]) K. W. DAMMERMAN, De Zoögeographie van Java. Handel. 3de Ned.-Ind. Natuurwet. Congr. 1925, p. 1–16 (sep.).

L. F. DE BEAUFORT, Zoögeographie van den Indischen Archipel. Haarlem, 1926.

K. W. DAMMERMAN, On the Zoogeography of Java. Treubia, Vol. 11, 1929, p. 1–88.

M. SANDERS, Die fossilen Fische der alttertiären Süsswasserablagerungen aus Mittel-Sumatra. Verh. Geol. Mijnb. Gen. Vol. 11, 1934, p. 1–143.

a few scattered land-patches of a certain elevation remaining emerged as relics of the lost territory.

This view involves that the establishing of the non-marine fauna in most of these islands goes back as far as the beginning of the Quarternary period.

The separation, however, of the faunules of these outlying islands from the fauna of Java dates from the centuries following the melting of the ice-sheets and the gradual regression of the sea. So if not other factors (e.g. transport by drift, birds, or human agitation) have affected the fauna, the population of these islands can be considered to be derived of that pre-glacial ancestry.

Only the Duizend Islands make an exception. As we have seen all of them are of a coralligenous nature and consequently their origin is tied to certain conditions of depth, temperature, density and grade of silt-content of the seawater. Some of the islands probably existed already at the beginning of the Glacial period, forming a fringing reef of the Soenda Land. Others, however, now lying farther into the Java Sea, could not develop until after the Ice Age, when, in the renewed Java Sea, conditions for coral growth were satisfactorily realised.

It is significant that the thousands of years which have passed since the Pleistocene epoch have not given rise to any new species at all. Only some local forms of minor importance: *Amphidromus contrarius baweanicus*, *A. perversus rufocinctus* and *A. perversus rufocinctus sankapurus* (all Bawean) have come to our knowledge.

The only site which has revealed some malacological novelties are the Kangean Islands. Here we have 4 endemic species: *Geophorus rollei*, *Cyclotus kangeanus*, *Hemiplecta kangeanensis* and *Landouria kangeanensis*, on a total of 7 land-shells.

After having discussed in the preceding lines the molluscs of the satellite islands off the West- and North-coast, and their historic and faunistic relations with the main island, we will now pass to the islands of the Southern Section: Noesa Baron, Noesa Kembangan, Trouwers Island and Klapper Island.

The extreme dissimilarity between the North- and South-coast of Java involves a corresponding unequality in the nature of the outlying islands. On the North side we have a flat coastal plain, gradually subsiding under the water surface till depths of 50 m on the average. The shore and the sea-bottom are rich in deposits of fine sand and mud.

On the South side, however, we are placed before a steep, cliffed coast, copiously provided with ridges, solitary rocks and sandflats, descending to the Indian Ocean in such a rapid slope that within a short distance depths till 3000, a little farther seaward even till 7000 m, can be sounded.

So, if we suppose that the island of Bawean, now the most advanced outpost of Java to the North, were lying at the same distance off the South-coast, it would not be surrounded by a shallow sea of circa 65 m depth, but by a profound of 3700 m.

This configuration of the submarine conditions off the South-coast implies that the formation of satellite islands is greatly handicapped here and can only be realised close to the shore.

From Noesa Baron a species and a variety of Clausiliidae: *Phaedusa moritzi* and *Ph. heldi* var. *baronensis* are recorded which are not found elsewhere. For the rest the land-shells belong to the ordinary Java species.

Noesa Kembangan is the most important of the South-coast islands. It has 9 true fresh-water and 19 landsnails (species of *Assiminea*, the *Auriculidae* and *Polymesoda* not counted), which are all common inhabitants of Java. The rich faunule – it is the second in richness, after Madoera – is not only a factor of its relatively large size, but also of the favourable natural conditions and the presence of calcareous soil.

Trouwers Island and Klapper Island are both inhabited by *Chloritis helicinoides*, a species only found in West Java and in some islands off its North- and South-coast.

VI. ECOLOGICAL VALUATION

The ecological relevancy which we are going to discuss in this chapter is the result of the interaction between the animal, [1]) and its environment. This result can be achieved in various ways, which can be chiefly classified according to two view-points: 1. as seen from the animal's angle and 2. as it would appear from the milieu circumstances.

To begin with the latter we touch upon favourable and detrimental features among the solid, liquid, living, dead, chemical or physical agents. A detailed analysis of the surroundings, however, is not, or only partly, possible, as only the minor

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[1]) Only land mollusca are considered here.

portion of the material, treated in this paper, was collected with special regard to ecological problems.

Altitude. Within the range of the Malay Archipelago the increase of altitude above sea-level generally involves an increase in moisture and a decrease of temperature. Of these conflicting elements the latter determines the final result and is liable for a diminishing of the snail population. Hence the mountain districts are remarkably poor in species and in individuals as compared with lowlands. This condition, it must be emphasized, is not in the first place caused by the limited extent of hilly and mountainous country in these miniature islands, but is most positively effected by the fall of the thermometer.

The following species are recorded from a certain elevation above sealevel: *Pupina superba* Pfr. (Sebesi) 700 m, *Succinea javanica* Schepm. *Opeas gracile* (Hutt.) and *Kaliella indifferens* Boettg. (all Krakatau) 100–200 m, *Helicarion lineolatus* Marts. and *Microparmarion strubelli* Simr. (both Sebesi) 700 m, *Amphidromus perversus rufocinctus* Fruhst. (Bawean) 250 m and *Amphidromus porcellanus* (Mouss.) (Krakatau) 500–800 m.

Lime. It is generally admitted that lime is an encouraging factor for mollusc development, not only for the animal's individual life, but also for the population of a certain habitat. Calcium is indispensable for the building of the shell and, in many cases, for the secretion of a few other vital parts: dart, egg-shell, epiphragma. Lime is assimilated either directly, by scraping off minute particles from the rock by means of the radula, or, more frequently, indirectly, by browsing and digesting the vegetation growing on calcareous soil, a diet rich in calcium-combinations.

In a large way the calcareousness of the surface soil can be read from the geological map. It brings to light that all the Duizend Islands, nearly the whole of Madoera, a large part of the Kangean Archipelago, Noesa Baron and several patches in Noesa Kembangan consist of limestone rock or its eroded products.

When opposing these calcareous islands against the noncalcareous ones (all the islands of the Western Section, Karimon Djawa Islands, Bawean, Trouwers Island and Klapper Island) and making a calculation of the landsnails and their occurrence in either group, we arrive at the following result. Of a total number of 88 species of land mollusca (of all islands combined)

69 are found in the lime-containing islands and 38 in those where lime is absent.

This is a striking contrast indeed, even if it be admitted that this statement is affected with various errors, such as the complete disregard of any elevation, of the distance to Java, of the capacity of each separate island and above all things the neglect of the influence of the vegetation.

Size of Habitat. It has already been incidentally remarked that large islands usually contain more species than small ones. Thus we saw that Madoera is inhabited by 34 species of land snails, Noesa Kembangan by 25. Other islands of the list in chapter IV more or less fully confirm this consideration.

The genial climate and the lime-content of the soil have been made partly responsible for this condition. Besides the mere size of the island in itself is a determinant factor, as large loci offer more possibilities for importation and settlement and a greater variety of habitats and subhabitats.

Large species, like *Cyclophorus*, *Hemiplecta*, *Amphidromus*, require more room than tiny or middle-sized species of *Gastrocopta*, *Opeas*, *Kaliella* and the like.

For the moment I will no longer insist on this point, as it is too much liable to discrepancies, because the method of collecting in the various islands has been so divergent that the results are hardly comparable.

Temperature. On the whole we can say that the rising of the temperature has a beneficient effect on snail life, unless it exceeds 30° C. Hence the lowlands in the Malay Archipelago are generally inhabited by a profuse snail population, premising that the favourable influence of the temperature is not overridden by harmful circumstances such as drought, absence of vegetation or too monotonous development of it, progress of cultivation, e.a.

Nearly all the islands discussed in this paper belong to the lowland zone (till 650 m) only a few tops in Krakatau, Sebesi and Bawean reaching into the hill zone (650–1500 m). As the temperature at sea-level is on the average 25° C and as it goes down circa ½° C for every 100 m elevation, it can be admitted that most of the molluscs treated in this paper live is a milieu varying between 25° and 22° C, only a few exceptional cases inhabiting some isolated stations where the thermometer sinks to 21° C.

Correspondingly there is little opportunity for comparing the

two regions and for checking the influence of the lower temper-
ature in heigher altitudes on snail life.

Moisture. In addition to the temperature the degree of
moisture is of paramount importance for the prosperity of mollusc
development. In this respect the satellite islands of Java are in
favourable circumstances, as they are lying in a part of the
globe with a very high amount of rainfall.

It has been observed in Java that the average number of
rain-days during the four consecutive driest months of the year,
for every station is producing the greatest controlling effect on
the vegetation and, consequently, on animal life, more than the
monthly or yearly average amount of rain can bring about.
(J. BOEREMA, Gemiddeld aantal regendagen op Java en Madoera
in de vier opeenvolgende, voor iedere plaats droogste maanden
van het jaar, – Verh. No. 23, Kon. Magn. en Meteorol. Observ.
Batavia, 1931, 25 pp., 1 map).

For the outlying islands, however, such figures are not
available so far. We can only dispose of the rain-gauge figures
for a few stations in Edam, Karimon Djawa Ids., Bawean,
Madoera and Kangean Ids. They are reproduced below,
together with the figures of the neighbouring stations in Java
which may serve for comparison (after J. BOEREMA, Regenval
in Nederlandsch-Indië. – Verh. No. 24, Kon. Magn. en Meteorol.
Observ. Batavia, 1931, Deel I, Gemiddelden van den regenval
voor 3293 waarnemingsplaatsen in Nederlandsch-Indië, bere-
kend uit waarnemingen verricht in het tijdvak 1879–1928).

	No. of years	Altitude	Average rain fall in mm	Average No. of rain days
Batavia (Observatory) . . .	50	7	1815	137.6
Tandjong Priok	29	0	1641	102.9
Edam Id. , ...	39	0	1621	85.5
Semarang (Bodjong)	50	2	2168	142.3
Karimon Djawa Ids. . . .	25	0	2073	142.2
Soerabaja (Oedjoeng) . . .	44	5	1559	112.9
Bawean Id. (Sangkapoera) .	29	25	2564	131.9
Madoera Id. (Pamekasan) .	50	15	1597	108.5
Kangean Ids. (Ardjasa) . .	32	20	2037	—
Kangean Ids. (Tambajangan)	12	130	2196	—

Although these figures cover only part of the islands mentioned in this report and are lamentably incomplete for the others, giving no evidence for the correctness of the thesis that the mean number of rain-days in the four driest months is the chief controlling factor for the regulation of vegetable and animal life, yet they do demonstrate that rainfall is plentiful in the islands surrounding Java. Hence it is not too rash to conclude that the relative air humidity will constantly be very high, a factor of prime necessity for a flourishing snail population.

Vegetation. The connection with the vegetation has been checked for a few cases only. Thus *Hemiplecta javacensis* and *Amphidromus perversus* were found in the Karimon Djawa Ids. in wood near the churchyard, and *Amphidromus perversus rufocinctus* was found in Bawean, on the stem of Sterculia foetida.

From what is known in other, similar, localities in the Malay Archipelago, however, we can in the main classify the landshells treated in this paper into two vegetation zones: the strand formation and the primeval forest of the hills, a lighter type of jungle which passes off as virgin forest in these islands. Only a few solitary instances are known of species penetrating into the higher forest region. Mangrove vegetation is rare in the islands surrounding Java; it can be entirely left out of account here.

The strand formation is partly composed of grass and other herbaceous plants, i.a. the well known Ipomaea pes-caprae, and of Pandanus- and coconut- palms and low shrubs, merging into a higher strand wood in which Chemara (Casuarina equisetifolia) and Barringtonia asiatica prevail.

In this strand formation molluscs occur nearly exclusively in the ground litter, between twigs and leaves. Although not exactly a type of vegetation, this is a kind of environment with highly specialized conditions of existence indeed, and as a rule it is densely populated. The snails frequenting it are generally small and not very conspicuous. Yet careful examination brings to light that, although their size is minute, their number is respectable. It is a favourable habitat for *Hydrocena javana*, *Allochroa conica*, *Auriculastra subula*, *Melampus fasciatus*, *M. luteus*, *M. variabilis*, *Cassidula auris-felis*, *C. labio*, *C. mustelina*, *Pythia castanea*, *P. chrysostoma*, *P. pantherina*, *P. plicata*, *P. scarabaeus*, *P. trigona*, *P. undata*, *Semperula maculata*, *Succinea gracilis*, *Tornatellina cylindrica*, *T. subcylindrica*, *Gastrocopta euryomphala*, *G. lyonsiana*, *G. pediculus ovatula*, *Subulina octona*, *Opeas gracile*, *Prosopeas achati-*

naceum, P. turriculum, Stenopylis coarctata, Kaliella doliolum, K. indifferens, Microcystina nana, Durgellina convexoconica, Landouria ciliocincta, L. rotatoria, L. winteriana, Chloritis helicinoides, Bradybaena similaris.

Almost the same remark holds good for the hill forest. Here again the casual visitor feels disappointed as to the abundance of land shells. Close examination, however, reveals a fairly rich snail population, living partly on the leaves and stems of trees, partly in the ground mould. This region is inhabited bij *Leptopoma vitreum, L. altum, Japonia trochulus, Cyclophorus perdix, Cyclotus corniculum, Succinea javanica, S. listeri, S. obesa, Hemiphaedusa cornea, Phaedusa corticina, Ph. heldi, Ph. javana, Ph. moritzi, Ph. sumatrana, Trochomorpha bicolor, T. planorbis, Eurybasis conus, E. multicarinata, Hemiplecta bataviana, H. humphreysiana, H. javacensis, Dyakia martini, D. rumphii, Helicarion adolfi, H. lineolatus, Landouria ciliocincta, L. rotatoria, Chloritis crassula, C. fruhstorferi, C. transversalis, Amphidromus contrarius* subsp. *baweanicus, A. filozonatus, A. heerianus* var. *poecilus, A. inversus, A. palaceus, A. perversus, A. porcellanus, Pseudopartula galericulum.*

Quantitative figures, however, cannot be given, as the samples which I received for examination had no indications of the units which they covered. Reference to the quantitative soil fauna investigations by DAMMERMAN will be made at the end of this paper.

Settling, Acclimatization. The members of the strand formation are at the same time the pioneer settlers in a new habitat. From observations made on the repopulation of Krakatau it has been ascertained that there exists a definite sequence with which faunal elements strike in a new locus (cfr. K. W. DAMMERMAN, Krakatau's New Fauna, in: Ch. E. STEHN, W. M. DOCTERS VAN LEEUWEN & K. W. DAMMERMAN, Krakatau. – Fourth Pacific Science Congress, 1929, p. 83 and 88).

First come the scavengers, next herbivors and finally carnivors and parasites.

In Krakatau the detritus dwellers still form the majority of the land snail colony, only one single species of the actual fauna *Amphidromus porcellanus*, being not bound to this ground biotope. Thus we see that as far as the molluscs are concerned, the new fauna has not risen very far above the initial stage.

Compared with the conditions in Krakatau the fauna of most of the other outlying islands of Java is equally in the same prima-

ry stage of population, only the larger islands like Bawean, Madoera, Noesa Kembangan and perhaps a few others having passed to the subsequent stage: the augmentation of the fauna by herbivors.

It remains to be seen whether we have to do here with an evolutionary stage of analogous tendency as in Krakatau. From what we heard before in this paragraph and the preceding one on the historical and ethological circumstances of the other outliers of Java there is strong evidence that the mere facts here are not directly comparable with such recent and relatively simple conditions as in Krakatau.

Another interesting phenomeon has been observed in the fauna of Krakatau, viz. that "the increase in the number of animal species is not in direct proportion to the passage of time" (K. W. DAMMERMAN. l.c. 1929, p. 90). This means that in the first decennia after the eruption the increase in number of species was more rapid than it is at present and than it will be in the future. The exactness of this thesis cannot satisfactorily be tested by the conditions in other islands of the present research, because we know almost nothing about the earlier mollusc stock of most of them, not even within the same lapse of time (round 50 years) since Krakatau started its new fauna. Indeed the present account is the very first inventory of the molluscs of most of the islands surrounding Java, with the exception of a few incidental records from Madoera, Kangean and Noesa Kembangan.

Dispersal, Competition. The detritus dwellers of the strand formation are on the whole common and widely distributed animals. This is amply demonstrated by the present study species like the *Auriculidae, Semperula maculata, Tornatellina cylindrica, T. subcylindrica, Gastrocopta euryomphala, G. lyonsiana, G. pediculus ovatula, Subulina octona, Opeas gracile, Prosopeas achatinaceum, P. turriculum, Stenopylis coarctata, Kaliella doliolum, K. indifferens, Microcystina nana, Durgellina convexoconica, Landouria rotatoria, L. winteriana, Bradybaena similaris* having a wide range of dispersal even outside the Malay Archipelago. In this respect there is no difference between Krakatau and the other satellite islands of Java.

In broad outline evidence all goes to show that there is a definite mutual relation between the faunal elements in a certain region. This relation is manifest from a systematic point of view in the ratio of Mammals, Birds, Reptiles, Fishes, Insects, Crus-

tacea, Molluscs, & c., but it is also demonstrated by the diet standard: carnivors, herbivors, scavengers.

In the case on hand there is no claim to any concurrence between the animal classes for the simple reason that nearly to nothing is known about animals other than some fishes and molluscs.

There is no preponderance of a certain species or a special taxonomic group over the other creatures in the same habitat, competition between land mollusca being of far less importance than it is between aquatic mollusca.

The only thing we can state is that the detritus fauna is better represented than any other class. As we have seen before this is partly a function of the evolutionary stage of the population (stage I) and partly of the physical conditions of the locality.

Food. Considering the feeding relations we have seen already that carnivors are lacking among snails. Herbivors and scavengers are represented in almost equal proportions.

The most important point of the animal's appreciation of its environment is lying in the food problem. Now there is almost nothing known about the diet of tropical molluscs. From the presence of a fairly rich snail fauna in nearly all the islands treated in this paper it can be concluded that the habitat produces at least such minimum quantities of food as suffice for the sustenance of a fairly good number of snails.

Of the three categories of animal nutrition: predatory, herbivorous or scavenging the Gastropods of the islands arond Java are in the main herbivors or scavengers, the very rare cases of carnivorous snails being not represented in these localities.

The herbivors are either feeding on leaves directly, or they are selecting minute algae, lichens or mould covering the stems and leaves of higher plants. The scavengers attack the vegetable debris and decaying leaves on the ground.

This latter category, the fauna of the vegetable litter on the bottom of the tropical jungle has been studied elaborately by DAMMERMAN for the Indo-Malay region. In several localities under various conditions he took samples of the layer of rubbish on the ground. For the method and for other particulars I must refer to DAMMERMAN's papers (K. W. DAMMERMAN, The Fauna of Krakatau, Verlaten Island and Sebesy. – Treubia, Vol. 3, 1922, p. 61–112; ID., First Contribution to a study of the Tropical Soil and Surface Fauna. – Ibid., Vol. 6, 1925, p.

107–139; ID., Second Contribution to a study of the Tropical Soil and Surface Fauna. – Ibid., Vol. 16, 1937, p. 121–147).

Among the islands which he investigated the following form also part of the present paper: Prinsen Island, Krakatau, Verlaten Island, Sebesi, Noordwachter, Zuidwachter, Klein Kombuis, Purmerend, Hoorn, Edam, Enkhuizen, Karimon Djawa and Bawean.

A comparison between the figures for species and individuals given by DAMMERMAN and the numbers in my account show some discrepancies, because the samples examined by me were not always the very same upon which DAMMERMAN's figures were based: they were either part of his samples or they were entirely fresh, parallel, collections taken at the same spot, but without considering the quantitative method.

A few points must be specially emphasized. DAMMERMAN mentioned some very high figures for the small ground dwelling snails in Klein Kombuis (Contribution Soil Fauna etc., 1925, p. 110 and 132). Here 5 samples were collected, 1 in the littoral zone and 4 in the virgin forest. The number of species was small in all cases (resp. 4, 4, 5, 3, 3), but the individuals were numerous (resp. 45, 106, 83, 96, 70).

My figures show the following: the 2 samples of July combined have 4 species with a very large number (not counted) of individuals, the 2 samples of November combined have 8 species with 158 individuals. The January sample contained 2 species, each with 1 specimen, and a sample taken August 1922 presented 1 species with 32 individuals. These results fully confirm DAMMERMAN's opinion that Klein Kombuis is a favourable habitat for small land mollusca.

Noordwachter, another small coral island, proved also to be a good collecting ground for surface dwelling snails. Here DAMMERMAN noticed the highest number of species per meter square and he took 9 species in each of his two samples (individuals not counted) (Contribution Soil Fauna etc., 1925, p. 123 and 131). According to my identifications there are 12 species in both samples combined, yielding a total number of 309 individuals. In this case again there is a general agreement with DAMMERMAN's statement, notwithstanding the fact that the mere figures are not exactly identical.

From the preceding description and discussion the importance of DAMMERMAN's admirable pioneer work is fully evident.

His conclusions would even have gained in completeness if he could have disposed at that time of the full data of the malacological results, a desire equally concerning other systematic groups. But, as DAMMERMAN more than once complained: "many of the species and even whole groups of animals ... remained unnamed, as ... the material collected consists of very small species or belongs to groups very hard to get identified by any specialist".

Therefore this report is, I hope, another proof for the absolute value of taxonomic work, both as an auxiliary science and as an independent discipline.

DIE ENTWICKLUNG DER SCHWIMMBLASE VON COREGONUS WARTMANNI

VON

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Beim Studium der Salmoniden-Schwimmblase hat man sich hauptsächlich beschränkt auf die Lachse, *Salmo salar* und *S. hucho*, und die Forellen, *Trutta fario* und *T. trutta*. Bekanntlich geht bei den genannten Fischen ein kurzer und gerader Ductus pneumaticus allmählich in den kranialen Teil der Schwimmblase über. Dieser Ductus entsteht bei diesen Salmoniden aus einem dorsalen Auswuchs des stark nach der rechten Seite geschobenen Darmes. Das dem Darme zugekehrte Ductusende weist jedoch, wie von STRICKER (1899) beschrieben und von MOSER (1903) bestätigt wurde, während der Entwicklung eine Verschiebung von der rechten nach der linken Seite auf. Diese rechtsseitige Lage des Darmes und der Schwimmblase wird bei den genannten Fischen von einer gewaltigen Dotteranhäufung verursacht. Während der Dotter allmählich aufgezehrt wird, dreht sich nach STRICKER und MOSER der Darm um seine Achse; der Ductusanfang verschiebt sich durch diese Bewegung nach der linken Seite und nimmt demzufolge eine Stelle unter, oder sogar etwas Links von der Chorda ein. MOSER und RAUTHER (1905) beschreiben die Schwimmblase als eine kegelförmige Fortsetzung des Ductus pneumaticus.

Die von diesen Untersuchern beschriebenen Beobachtungen kann ich auf Grund meiner Wahrnehmungen im Amsterdamer Laboratorium bestätigen. Von *Trutta trutta* standen mir Jungfische von 13,8, 17,7 und 21 mm zur Verfügung. Beim jüngsten Exemplar lag der caudalwärts gerichtete Schwimmblasenknopf mit kleinem ̤umen rechts oberhalb des Darmes. Der Fisch von 17,7 mm zeigte dieselbe Lage, aber wies Faltung des Ductusepithels und Fortsetzung dieser Falten im vorderen Teil der Schwimmblaseninnenwand auf. Im nächsten Stadium, beim Fisch von 21 mm, hatte sich der Ductusanfang nach der linken

Darmwand verschoben. Der Luftgang liegt jetzt auf der Lateral-
seite des Darmes und setzt sich in einigen Schnitten kaudal fort,
biegt dann unter der Vorderspitze der Schwimmblase um und
geht links ventral in sie über.

Während *Salmo* und *Trutta* öfters zur Untersuchung der
Schwimmblase benutzt wurden, hat man die Schwimmblasen-
entwicklung bei *Coregonus*-Arten noch nie ausführlich beschrie-
ben. In VOGTS „Histoire naturelle des Poissons d'eau douce''
(1842) wird *Coregonus palaea* genannt. Der Verfasser beschreibt
wie hier bei jüngeren Embryonen der Schwimmblasenknopf aus
dem Darm in der Nähe des Magens entsteht und wie diese Blase
erst 2–3 Wochen nach dem Ausschlüpfen zu funktionieren an-
fängt. DE BEAUFORT nennt *Coregonus lavaretus* und *C. oxyrrhynchus*
in seiner Dissertation, gibt aber nur Mitteilungen welche sich
auf erwachsene Fische beziehen. Vom Salmonidentypus ab-
weichende Besonderheiten werden nicht erwähnt.
  Ich konnte die im Zoologischen Institut vorhandenen Schnitt-
serien von *Coregonus Wartmanni* untersuchen. Das Material
stammte aus dem Institut für Seenforschung und Seenbewirt-
schaftung Langenargen am Bodensee, und war in Amsterdam
geschnitten worden; es eignete sich sehr für das Studium der
Schwimmblase.
  Ich benutzte 16 quergeschnittene Serien von Fischen, deren
Grösse variierte von 18–73 mm.
  Bei den 5 kleinsten Fischen, von denen mir vollständige
Schnittserien vorlagen, konnte festgestellt werden, dass der An-
fang des Ductus sich unter Wirbel acht befindet. Mutmasslich
nimmt der Ursprung des Luftganges bei *Coregonus Wartmanni*
auch im späteren Alter in Bezug auf die Wirbelsäule immer
denselben Platz ein. Die Stelle, wo sich der Ductus in kaudaler
Richtung aus der Darmmuskulatur befreit, liegt kurz vor der
Übergangsregion des Darmes in den Magen. Die Wand des
Luftganges besteht aus einer Epithel- und einer Muskelschicht,
beide sind Fortsetzungen der gleichnamigen Schichten des
Darmes. Ringsumher liegt schliesslich noch eine dünne mesen-
chymatöse Masse, worin sich einzelne Blutkapillaren befinden,
die sich den äusseren Muskelzellen anlegen.
  Der Ductus entspringt auf der Dorsalseite des stark nach
rechts verschobenen Oesophagus. Dieses an den Darm grenzende
Ende zeigt bei den aufeinanderfolgenden jüngeren Stadien (18,

21 und 27 mm) eine kleine Verschiebung nach der Medianlinie des Körpers hin.

Man findet bei dieser *Coregonus*-Art nicht den einfachen, kurzen Ductus pneumaticus, welcher andere Salmoniden kennzeichnet. Im Gegenteil, der Luftgang ist hier ein langgedehntes, bei älteren Fischen sogar umgeknicktes, Röhrchen, dessen Lage sehr verschieden sein kann.

Um eine einfache Übersicht des Ductusweges zu ermöglichen habe ich nach den Präparaten Rekonstruktionen angefertigt (Abb. 5) und folgendes Schema aufgestellt:

A. Der lange und gerade Ductus pneumaticus, mit gefalteter Epithelschicht, geht ohne scharfe Grenzen in die Schwimmblase über (18 mm).

B. Es bildet sich eine doppelte Umbiegung des Luftganges. Der Teil, der an die Schwimmblase grenzt, verbreitert sich und geht allmählich in die Schwimmblasen-vorderspitze über (21 und 28 mm).

C. Der Ductus ist nur einmal geknickt. Die Vesica natatoria hat sich kopfwärts stark ausgedehnt. Die kranial verlaufende Röhre des Luftganges endigt vorn in der Schwimmblase (30, 33 und 47 mm).

Wir nennen das Stück des Ductus, das in den Darm mündet den „intestinalen" Teil und das in der Nähe der Schwimmblase befindliche Stück des Ductus den „vesicären" Teil (DE JONG, 1936).

Die Fische von 18 und 27 mm besitzen den unter A genannten allmählichen Übergang des Ductus in die Schwimmblase. Das über die ganze Länge gefaltete Epithel des Luftganges setzt sich, bei diesen Blaufelchen wie bei den Forellen, auf der Schwimmblaseninnenwand fort und geht da in flaches Epithel über. Diese kleinen Fische haben also den gewöhnlichen Salmoniden-Typus.

In den folgenden Stadien nehmen die Vorderspitze der Schwimmblase und der jetzt geknickte Ductus stark in Länge zu. Der von denselben in der Leibeshöhle eingenommene Platz ist unter Einfluss der wechselnden Lage der Verdauungsorgane sehr verschieden. Besonders der Ductus pneumaticus wird in den Schnittserien häufig an immer wieder anderen Stellen gefunden.

Ich glaube, dass der Druck, der bei Verschiebung des Darmes in dem vorderen Teil der Leibeshöhle auf die Schwimmblasenanlage ausgeübt wird, als die Ursache angenommen werden

muss, wodurch bei *Coregonus Wartmanni* während der Entwicklung der Weg des Ductus zu wiederholten Malen abgeändert wird.

Die zweimalige Knickung des Ductus (Gruppe B) tritt zum ersten Male beim Fisch von 21 mm auf. Dieser hat im Vergleich zu dem Exemplar von 27 mm eine bereits weit entwickelte Schwimmblase. Man findet dieselbe direkt hinter dem „Magen-knie". Durch diese Lage wird die Blase anfänglich in seiner vor-wärts-gerichteten Entwicklung gehemmt. Die Knickung ist noch sehr schwach und liegt im Bereich des 12. und des Anfanges des 13. Wirbels. Unter den Nieren ist in diesem Falle in der Leibes-höhle Raum genug, man findet in den Schnitten die drei Teile des Luftganges nebeneinan-der (Abb. 1).
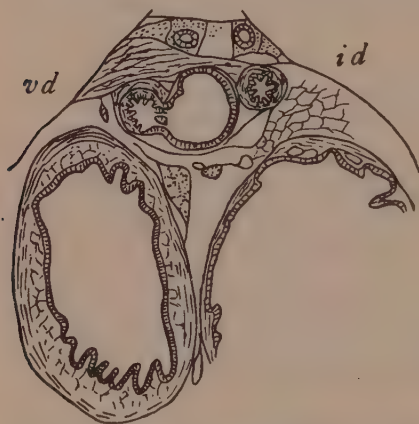
Abb. 1. *Coregonus Wartmanni*, 21 mm: Die drei Teile des geknickten Luft-ganges liegen nebeneinander. *id*, intestinaler Teil des Ductus; *vd*, vesicärer Teil des Ductus. Querschnitt, Vergr. × 37.

Das Blaufelchen von 28 mm besitzt eine Schwimm-blase, die sich schon ein Stück dorsal vom Magen kopfwärts ausgebreitet hat. Die intesti-nale Röhre des Ductus läuft zuerst kaudalwärts, biegt dann, in einer senkrechten Ebene, in dorsale Richtung um, und geht in eine dem Kopf zu laufende Röhre über; die Umbiegung dieses kranial verlaufenden Teiles in den wieder kaudalwärts zu folgenden vesicären Teil des Ductus geht jedoch nach rechts und liegt in einer hori-zontalen Ebene (Abb. 2). Die intestinale Knickung liegt in einem kleinen Raum zwischen dem Oberrand des Magens einerseits und dem Mitteldarm anderseits. Weil der Mitteldarm sich dorsal beinahe bis an das Peritoneum verschoben hat, konnte der vesicäre Ductusgang nur stark rechts biegen und kam infolgedessen dorsal vom Magen zu liegen. Nachdem sich der Ductus aus der Darmmuskulatur (am Ende des 8. Wirbels) frei gemacht hat, dehnt sich das intestinale Ende, innen mit einer flachen Epithelschicht bekleidet, bis unter den 11. Wirbel nach hinten aus, biegt dann um und läuft, weiter überall mit einem gefalteten Epithel versehen nach vorn

(bis Anfang des 9. Wirbels), wendet sich dann wiederum dem Schwanz zu und geht (erst unter dem 10. Wirbel) allmählich in die Schwimmblase über.

Die Schwimmblase der grösse-ren Fische hat sich dem Kopf zu stark ausgedehnt. Wie oben in Gruppe C angegeben wurde, läuft der kranialgerichtete Teil des Ductus nun direkt ohne sich wieder nach hinten umzubiegen vorn in die Schwimmblase aus und bildet also das vesicäre Ende.

· Beim Fisch von 30 mm hat sich das Lebergewebe stark entwickelt und auch die Lage des Darmes geändert. Die Speiseröhre und deren Fortsetzung in die Magen-gegend liegt hoch, direkt unter dem Peritoneum; der Mitteldarm dagegen ist nun stark ventral gerückt. Die weit kopfwärts ver-schobene Schwimmblasen-Spitze wurde seitwärts gedrückt und liegt nun dorsal des Mitteldarmes. Betrachten wir nun den Luftgang: Das intestinale Ende läuft in der Medianfläche nach hinten
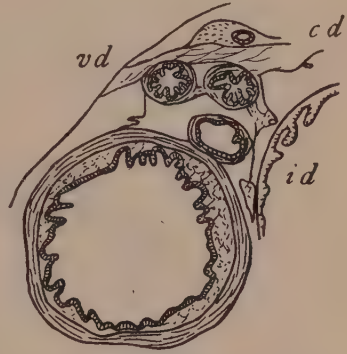
Abb. 2. *Coregonus Wartmanni*, 28 mm: Der Mitteldarm reicht beinahe bis an das Peritoneum, die drei Teile des Ductus pneu-maticus liegen dorsal des Magens. *id*, intestinaler Teil; *cd*, krania-ler Teil; und *vd* vesicärer Teil des Ductus. Querschnitt, Vergr. × 37.

und ventralwärts und biegt unter der Mitte des 10. Wirbels nach vorn und ventralwärts um. Dieser kranial-gerichtete, vesicäre Teil des Ductus geht, ohne weiteres Knicken, vorn rechts ventral (unter dem 6. Wirbel) in die links gelegene Schwimmblasenvor-derspitze über (Abb. 3). Wie oben hat auch hier der intestinale Teil ein glattes und der
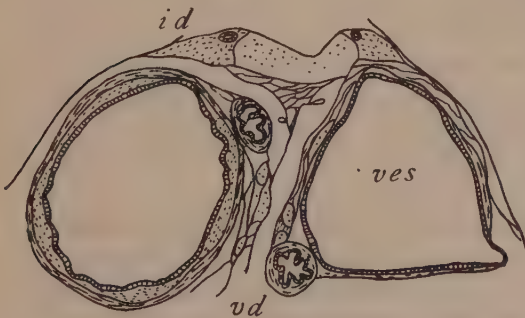
Abb. 3. *Coregonus Wartmanni*, 30 mm: Der Ductus pneumaticus geht rechts ventral in die Schwimmblasenvorderspitze über. *id*, intesti-naler Teil; *vd*, vesicärer Teil des Ductus; *Ves*, Schwimmblase. Querschnitt. Vergr. × 37.

vesicäre Teil ein gefaltetes Epithel. In diesem Fall lag die
Schwimmblasenspitze ausnahmsweise dorsal des Mitteldarmes.
In den folgenden Serien liegt der Anfang der Schwimmblase
aufs neue entweder dorsal des Magens oder, wegen dem Vorwärts-
rücken der Vesica, dorsal des Oesophagus. Er muss also wiederum
ganz zurückverschoben sein, was auch von Einfluss auf den
Ductus sein muss.

Abb. 4. *Coregonus Wartmanni*, 47 mm: Das vesicäre Ende
des Ductus pneumaticus läuft links an der Seite in die
Schwimmblasenvorderspitze aus. *id*, intestinaler Teil;
vd, vesicärer Teil des Ductus; *Ves*, Schwimmblase.
Querschnitt, Vergr. × 37.

Betrachtet man jedoch den Verlauf des Ductus bei den grösse-
ren Fischen, dann sieht man keine wichtigen Änderungen mehr,
so dass der Ductus schliesslich seinen endgültigen Platz einge-
nommen haben muss. Die noch zu besprechenden Serien kann
ich also kurz zusammenfassen. Die untersuchten Fische von
33, 37, 42 und 43 mm besitzen einen intestinalen Ductus pneu-
maticus in der Medianebene des Körpers. Dieser Teil der Luft-
röhre sinkt weiter kaudal immer mehr an der Darmmuskulatur

ventralwärts, dreht sich dann stark links um, biegt dem Kopf zu wieder allmählich dorsal und geht schliesslich an der Unterseite links in die Schwimmblase über.

Die ältesten Stadien zeigen das intestinale Ende des Ductus neben, oder auch über dem Darm, das kranial gerichtete vesicäre Ende läuft auch wieder links ventral, oder, wie bei den Serien von 47, 65 und 73 mm, links an der Seite in die Schwimmblasenvorderspitze aus (Abb. 4).

Die Speiseröhre liegt bei älteren Fischen mehr der Ventralseite genähert als dem Mitteldarm. In dem lockeren mesenchymatösen Gewebe, das sich zwischen Nieren und Oesophagus findet, ist genügend Raum für die vordere Hälfte der Schwimmblase da. Für den Ductus aber ist der noch übrige Raum, zwischen Verdauungsorganen und Schwimmblase, sehr beschränkt. Die Epithelschicht des Luftganges hat sich, anders als bei den Fischen von 21, 28 und 30 mm, überall in Falten gelegt, was, meines Erachtens, nur als die Folge des Druckes der ringsum liegenden Organe betrachtet werden kann. Die Schwimmblasenspitze hat bei den grösseren Fischen, durch den Ausbau nach vorn, einen Punkt vor dem Ductusanfang erreicht. Nur in zwei Fällen, nämlich beim Fisch von 37 mm und bei einem der Tiere von 43 mm, fängt die Schwimmblase erst hinter der Stelle an, wo sich der Ductus aus dem Darm frei macht. In beiden Fällen liegt der Oesophagus hier noch ziemlich nah an den Nieren, so dass die Schwimmblase sich nicht dem Kopf zu hat ausbreiten können.

Typisch waren die Schnitte eines Blaufelchens von 62 mm Länge. Die pylorischen Anhänge am Ende des Magens sind hier sehr stark entwickelt, sie drücken den Magen und den Mitteldarm an die Seitenwände des Körpers. Die Ductusumbiegung lag in diesem Fall ungefähr median dorsal der genannten Anhänge.

Die beschriebenen, aufeinander folgenden Stadien machen es klar, welchen Einfluss die Bewegung des Darmes auf den vorderen Teil der Schwimmblase und des Ductus hat. Die grössten Veränderungen treten auf bei den Coregonen, deren Massverhältnisse zwischen 21 und 33 mm liegen. Sie werden hier, als Erklärung der Abbildung (Abb. 5), noch einmal kurz resumiert.

Die Knickung des Ductus bildet sich beim Fisch von 21 mm dadurch, dass die Schwimmblase in ihrem frontalen Zuwachs

aller Wahrscheinlichkeit nach von der Magenbildung gehindert wird. Später dringt die Schwimmblase dennoch rechts oben in der Leibeshöhle langsam vor und legt sich, weil der Darm nun etwas ventralwärts gedrückt wird, mit dem vesicären Ende höher als mit dem nach hinten ausgewachsenen intestinalen Ductus-Ende. Dies hat ein sich nach dorsal Umbiegen und
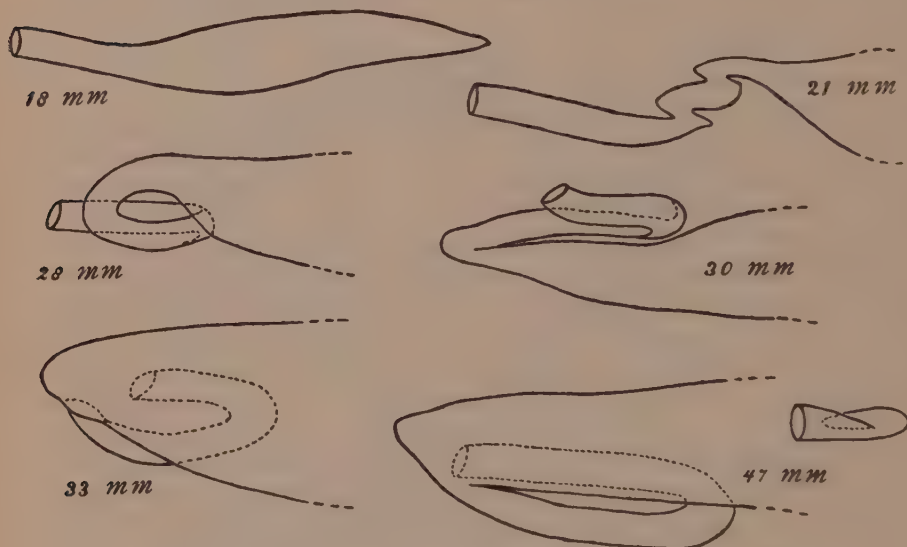
Abb. 5. Nach den Praeparaten angefertigte Rekonstruktionen des Ductus, mit dem Zweck die Veränderungen der Röhre während der Entwicklung deutlich zu machen. Von dem Fisch von 18 mm wurde die ganze Schwimmblase gezeichnet; von den Fisch von 21, 28, 30 und 33 mm nur die Vorderspitze, und von dem Fisch von 47 mm die Spitze und das Schwimmblasenende. Vergr. × 27.

kopfwärts Ausbreiten des Ductus zur Folge. Darauf wird die ganze Schwimmblasenspitze, beim Fisch von 30 mm, während die Speiseröhre sich etwas hebt, von der rechten Körperhälfte nach der linken Körperhälfte geschoben. Der nun zugleich vesicäre, kranialverlaufende Teil des Ductus pneumaticus wird beim Verschieben hinsichtlich des intestinalen Teiles etwas hinabgedrückt, wodurch eine Biegung des Ductus ventralwärts entsteht. Dass der Ductus jetzt vorn etwas mehr rechts mündet, ist eine Folge davon, dass die Schwimmblase sich kranial ausgebreitet hat. Der Oesophagus sinkt nun aber noch einmal nach

der Ventralseite des Körpers, während umgekehrt der daneben liegende Mitteldarm wieder dorsal steigt. Die Vorderspitze der Schwimmblase muss nun wieder etwas emporrücken. Dabei wird der intestinale Teil des Ductus nach ventral gedrückt und der vesicäre Teil dorsalwärts gehoben. Die Schwimmblase legt sich dann auf den Ductus. Letzterer bildet eine Schleife und mündet vorn links in die Unterseite der Schwimmblase aus. Endlich haben die Organe nun ihren endgültigen Platz in der Leibeshöhle gefunden, denn bei den Fischen von 37 mm und grösser treten keine bedeutenden Verschiebungen mehr auf.

Den Verlauf des Ductus ausgenommen, findet man bei den *Coregonus*-Schwimmblasen keine wichtigen Unterschiede mit den andern Salmoniden-Schwimmblasen vor. Für den weiteren Bau der Schwimmblase genügt daher eine sehr kurze Beschreibung.

Vorn in der Leibeshöhle ist der Raum, in dem sich die Schwimmblase seitwärts ausbreiten kann, beschränkt. Ihr Durchschnitt ist deshalb klein und, wie man bei allen Salmoniden-Schwimmblasen beobachten kann, ihre Innenbekleidung stark gefaltet. Sobald der Darm aber eine mehr ventrale Lage einnimmt, wächst der Diameter der Schwimmblase und füllt eine grosse dünnwandige sackförmige Schwimmblase in allen Stadien den Raum unter den Harnleitern. Das Ende der Schwimmblase ist immer rôhrenförmig und liegt, in einer mesenchymatösen Gewebemasse, rechts dorsal in der Leibeshöhle. Wie am Anfang ist auch am Ende der Schwimmblase die Epithelschicht stark gefaltet. Eine Muskelschicht hat sich um das Ende noch nicht gebildet. Eine kompakte – und lockere – mesenchymatöse Masse umgibt die Epithelzellen.

Die kaudale Schwimmblasenspitze zeigt bei den Fischen von 42 bis 65 mm eine Umbiegung, welche bei den kleineren Exemplaren noch fehlt, aber merkwürdigerweise auch beim Fisch von 73 mm nicht vorkommt. Das kopfwärts gerichtete Stück dieses Blindschlauches – es wurde von mir achtmal angetroffen – verlief sechsmal in der vertikalen und zweimal in der horizontalen Ebene. Dieses Schwimmblasenende erinnert an den blindschlauchartigen Anhang, der von RAUTHER und TRACY bei den Syngnathiden am Schwimmblasenende angetroffen wurde. Bei *Syngnathus*-Embryonen mündet hier der Ductus pneumaticus, also kann dieser kleine Zapfen am Ende der Schwimmblase ein Rest des embryonalen Luftganges sein. Diese Bedeutung kann der

Blindschlauch beim *Coregonus Wartmanni* nicht haben, weil da der Luftgang fortwährend bestehen bleibt. Unentschieden bleibe, ob das Zäpfchen bei *Coregonus* einige Bedeutung hat.

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THE ALIMENTARY CANAL OF CONTARINIA TORQUENS DE M. WITH SPECIAL REFERENCE TO THE HIND-GUT

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In the course of the last four years the distribution, development, and life habits of *Contarinia torquens* de M., a gall midge of the family Itonididae (Cecidomyidae) have been the subject of an extensive investigation by Dr. S. LEEFMANS in order to find measures for controlling this insect, which is a serious pest to the cabbage culture in causing a disease, known as "draaihartigheid", which may give rise to heavy losses.

The problems offered by the feeding and digestion of these larvae cannot be understood without some knowledge of their structure and life habits. A short account, therefore, of what is known, up to now, about the external morphology of the larva and about its mode of life precedes here. For more extensive information the reader should consult LEEFMAN's papers.

The larvae of *Contarinia torquens*, measuring in the last stage about 3 mm, are, like many other Itonidid larvae, very much modified, and almost maggot-like. They are relatively slender and somewhat depressed, i.e. broader than high. A breastbone (spatula sternalis) is always found in the older larvae and seems to be wanting in the very young ones only. Otherwise there are no appendages, except two very small antennae. The mouth parts are highly reduced, so that I have not been able to identify them.

These larvae live in small numbers together in the leaf axils of the young cabbage plants, causing a typical inflation of the bases of the leaf stems. In consequence of this deformation the young leaves are more or less hemmed in, and arrested in their development.

So, the larvae live on the outside of the hostplant and there is no question about their boring into its tissues.

All the same the plant may be severely injured, and that not

so much by the withdrawal of juices (in what way this probably is effected will be shown later on) as by the rot which often arises secondarily, killing the very hart of the plant. Very often, however, after some time, especially in the more resistent varieties, the plant overcomes the disease and recovers more or less completely.

One of the problems most puzzling Dr. LEEFMANS from the beginning, was the feeding of the larvae. As mere observation of the living larvae appeared not to give satisfactory information, it was considered useful to tackle the problem from another side, viz., by means of an anatomical and histological examination of the digestive system.

It fell to the part of the author to do this examination, the results of which are given in the present paper.

Among the extensive literature on the larval intestine of Diptera, there are several papers dealing especially with the Itonididae. Mention should be made of the papers of WILLIAMS (1910), ANDERSON (1935), METCALFE (1933), PURNENDU SEN (1938), and WEHRMEISTER (1925), those of the first mentioned four authors dealing with one species each, of which a more or less complete monograph is given, while the paper of WEHRMEISTER gives a more comparative survey of the larval gut in several genera and species.

It soon appeared, however, that the digestive system of the *Contarinia* larva agrees only to a certain extent with that of other species. Especially the relative dimensions are very peculiar here. This, and the circumstance that the hind-gut is rather modified seem to justify a detailed description of the whole digestive system of this remarkable larva.

Methods

The greater part of the anatomical examination has been done by means of series of sections of 5–7 μ.

The larvae, after being narcotised with aethyl-acetate, were fixed in Carnoy's fluid and embedded quickly in paraffin, since the method of double-embedding in celloidin and paraffin, generally considered more delicate, turned out to cause a considerable shrivelling in this particular case.

The sections were double-stained with Ehrlich's or Heidenhain's haematoxylin and eosin.
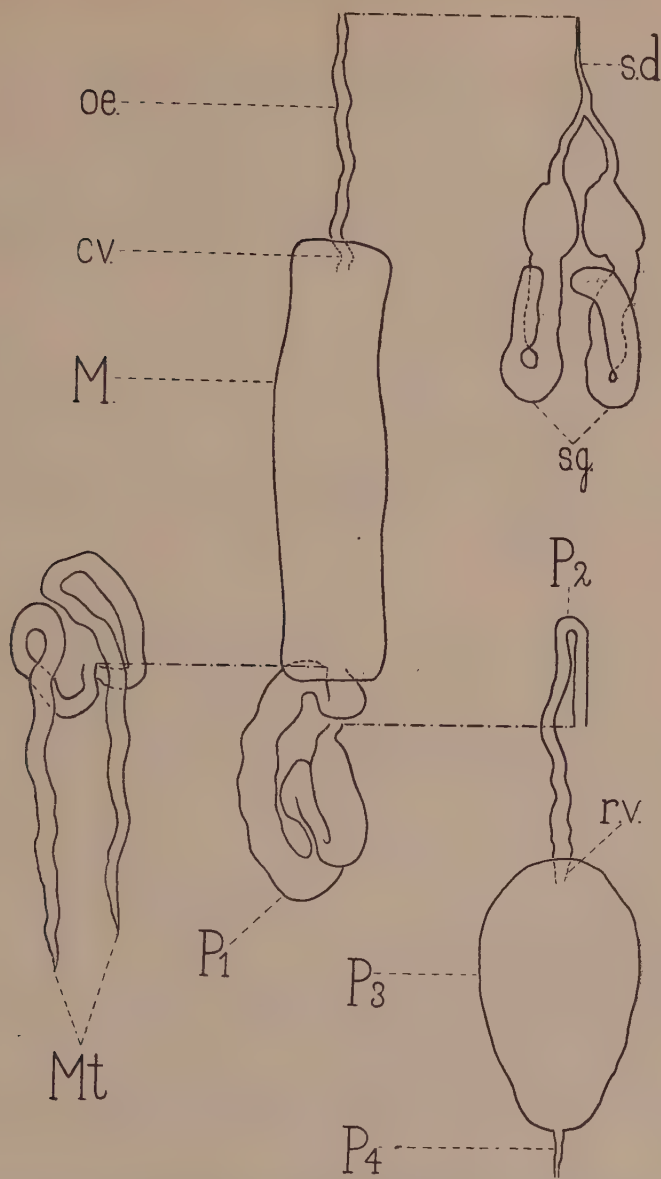
Fig. 1. Reconstruction of the intestinal tract of *Contarinia torquens* de M. Salivary glands, Malpighian tubes, and part of the proctodaeum have been moved to the right and to the left. Abbreviations: oe., oesophagus; c.v., cardiac valve; M, mesenteron; s.d., salivary duct; s.g., salivary glands; r.v., rectal valve; M.t., Malpighian tubes; P1, P2, P3, P4, parts of the proctodaeum.

Of course, it was also attempted to gain a general survey of the whole gut by dissection of the fresh larvae, but this effort has been only partly successful, the least incision causing the internal organs to protrude in a confused mass, and preventing, therefore, an accurate study of the situs. On the other hand, the extensive fat-body left visible only a small part of the tract in the intact larvae, except in the very young ones. The drawing, therefore, reproduced in Fig. 1, was made largely by means of reconstruction of serial sections.

Finally, attention should be drawn to the fact that, owing to the impossibility of discerning exactly the several stages, it has not been possible to indicate the specimens studied otherwise than by their length and the development of the fat-body. Therefore, in our terminology, "old" larvae are specimens of maximal length and with a fully developed fat-body, and "young" ones are specimens of $1-1\frac{1}{2}$ mm and with a still incomplete fat-body.

General aspect of the digestive system

Inspecting the intestine as a whole, it is seen at once that the division in the three main parts: stomodaeum, mesenteron, and proctodaeum, is quite obvious. The very thin fore-gut changes abruptly into the mid-gut, and the partition between this and the hind-gut, besides being marked by the origin of the Malpighian tubes, is hardly less evident.

Both the fore-gut and the mesenteron run straight through the body cavity, without a single curve or convolution. On the other hand, especially in its foremost part, the relatively very voluminous hind-gut is strongly convoluted, showing, moreover, a distinct subdivision.

The appendices of the digestive tract consist of a pair of strongly developed salivary glands and two Malpighian tubes, also of a considerable length.

The digestive tract and its appendages fill up the greater part of the body cavity; especially between the mid-intestine and the body wall, there remains only a small space.

The stomodaeum and the salivary glands

The very slender stomodaeum, which measures about 1/5 of the body length, shows, except in its foremost part, over the whole length the same character, and should, according to its

structure, be considered an oesophagus. Croplike dilations and proventricular thickenings are wanting altogether.

The wall, especially in the hindmost part, is rather thin and consists essentially of a low epithelium lined with a hardly visible cuticula and covered perhaps by a very thin muscle layer. More forward the wall grows stronger, both the cuticula and the muscle layer becoming thicker here, so that, the outer diameter being about the same as more backwards, the lumen becomes rather narrow.

The very foremost part of the stomodaeum (the pharyngeal cavity of the authors) is modified into a sucking pump. The wall of this part is very muscular and the cuticula, being also much more developed than elsewhere in the gut, encloses a strongly depressed lumen (Fig. 2). In the centre of the impression two muscles insert, which, diverging dorsad, probably constitute the dilating mechanism of the pump. In my opinion, there can hardly be any doubt as to the nature of this apparatus that evidently

Fig. 2. Cross section through the fore-most part of the body, showing the sucking pump. Abbreviations: c.l., chitinous lining of the pharynx; l, lumen of the pharynx; m., dilatory muscles; b.c., body cavity, filled with muscle tissue; b.w., body wall.

is just as much adapted to the sucking up of liquid food as the sucking pump of mosquitoes, flies, and Homoptera. This is an important point, to which we shall have to revert when discussing the function of the digestive system and the way of feeding.

The end of the fore-gut is invaginated into the mid-intestine, thus constituting a simple cardiac valve (Fig. 1). Any differentiation, however, of this part into a proventriculus, is out of the question (cf. METCALFE, ANDERSON a.o.).

The salivary glands consist of three distinct parts. viz., the salivary duct, a short and very thick glandular part, and a long tubiform division, curved forward about the middle. The two salivary ducts unite at the level of the sternal spatula into a single tube, which remains visible as far as the front of the sucking pump (Fig. 1).

The "second divisions" of both sides occupy together the greater part of the body cavity immediately in front of the mid-gut.

The third, or tubular division runs more or less close along the wall of the mesenteron; in Fig. 1 both the salivary glands have been turned somewhat round their axes in order to demonstrate the turning back. In reality the recurrent part lies dorsal to the descending part. Histologically the three parts are very different, whereas the lumen throughout the whole gland has about the same diameter which is very small.
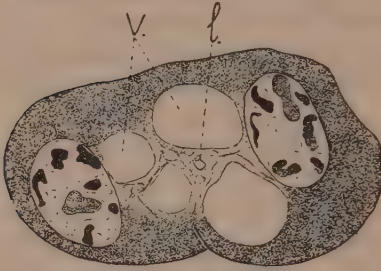
Fig. 3. Cross section through the tubular part of a salivary gland. Abbreviations: l., lumen of the salivary gland; v., vacuoles.

The tubiform part consists of rather large cells, never more than two of them being visible in the same section. Often, there is only one nucleus to be seen, just as in the Malpighian tubes. The nuclei do not show any peculiarities, but the protoplasm is
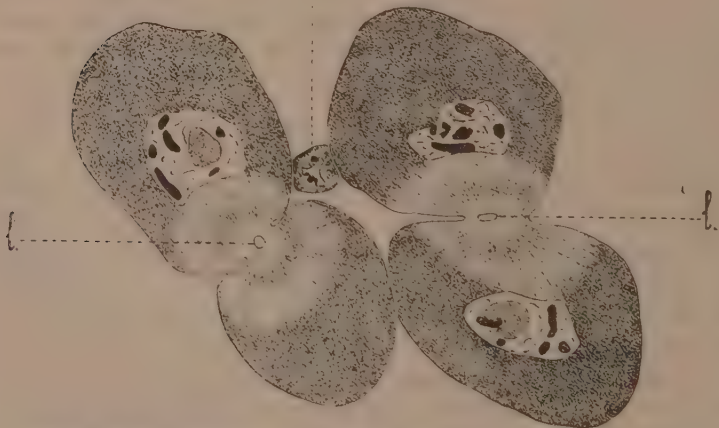
Fig. 4. Cross section through the globular part of the salivary glands and the oesophagus. Abbreviations: oe., oesophagus; l., lumen of salivary glands.

heavily vacuolised, the greater part of every section of the gland being occupied by vacuoles (Fig. 3). In several sections the content of these vacuoles has been stained red by eosin. Generally, however, the vacuoles are empty in the preparations, the contents evidently having been washed out in the course of the treatment.

The globular second part of the salivary glands is modified in a very particular way. Far from being a thin-walled reservoir as one might suppose when dissecting a larva (perhaps WEHR-MEISTER made this mistake) it consists of two very big cells, supplemented in front by several smaller ones of the same type. The dimension of these cells, in relation to the lumen, is to be seen in Fig. 4.

The large cells as well as the smaller ones are very different from the cells of the tubular part of the gland. Vacuoles seem to be quite lacking, the structure of the protoplasm being, on the contrary, very compact. In all my preparations they are stained intensely by haematoxylin. The plasma bordering the lumen shows a radial striation and, moreover, stains considerably more red than the peripheral part of the cell (Fig. 4).

The nucleus is voluminous and has a large nucleolus showing, for the rest, well delimited masses of chromatin representing possibly the giant chromosomes of the authors.

As to the probable functional meaning of the division of the glandular tissue into two parts, I am not prepared to give any suggestions.

The Mid-gut

As stated above, the mesenteron constitutes the most voluminous part of the digestive system, especially as regards the width. In many specimens, the mid-gut, being over its whole length of the same bulk, fills up nearly the whole of the body cavity.

The wall of the mesenteron consists chiefly of a magnocellular epithelium, showing, probably in connection with the respective phases of its activity, a rather varying aspect. The greater part, however, looks as in Fig. 5. Generally in transverse sections the whole wall is seen to be constituted by only 9–11 large cells. In my preparations the muscularis is hardly visible, but this may be due to the staining methods. What little is to be seen here and there suggests a network of muscle fibres. Anyhow, the peristaltic movements are very obvious and strong, as may be ob-

served in young larvae with a still incomplete fat-body. In many cases one or more peristaltic contractions have been fixed and are found in the sections as constrictions of the lumen.

Fig. 5. Cross section through the region of the mid-gut, showing i.a. the dark-stained contents of the mesenteron. Abbreviations: d.v., dorsal vessel; s.g., salivary gland; m.g., mid-gut; v.n.c., ventral nerve cord.

Our simple method, of course, allowed but a superficial study of the epithelium and its activities. Nevertheless, several conditions of the epithelium could be distinguished.

Taking the state, depicted in Fig. 5 for a standard, we first of all perceive that the proto-plasm seems to be of a fairly homogeneous constitution. Almost over the whole cell it shows the same fine granulation, in which vacuoles are altogether wanting. Generally, the nucleus occupies a central or somewhat more basal position. It seems to offer no peculiarities.
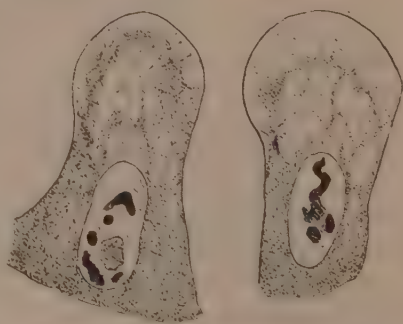
Fig. 6. Two cells of the mid-gut in the "high" phase.

This "standard cell" is liable to variation in two opposite directions. On the one hand there are sections in which cells are met with which are much higher, so as to constitute a very high epithelium, with cells projecting into the lumen of the gut. In this case the upper part of the protoplasm is considerably less stained and distinctly vacuolised (Fig. 6). Of course it is not possible to say with certainty whether the content of these vacuoles consists of the secretion fluid or of the food just resorbed. I prefer, however, the former interpretation, this type being particularly well represented in preparations which show the opposite variation too. This variation is constituted by extremely flat cells offering only just enough room for the nucleus. In our line of thought, these are cells which, having just poured out their secretion into the gut cavity, represent the last phase of excretory activity. I am well aware that a much more extensive study would be necessary to solve this problem, but think it expedient to record such data as I have been able to find.

There are two more details of which I am fairly sure.

First, the secretion seems to occur without the rejection of parts of the cell body itself, because in that event fragments should be found in the cavity of the mesenteron where indeed they are always wanting. So, a more or less complete degeneration and regeneration of whole cells, such as is found in several other insects, is altogether out of the question. This explains, in its turn, the total absence of anything like regeneration cells in the mid-gut of the *Contarinia* larva.

Then, something should be said about the distribution of the several stages of cellular activity along the mid-gut. According to WEBER a.o. there are three possibilities, first that of rhythmical activity of the whole epithelium, in which case every preparation shows one cell type only, secondly rhythmical secretion of a more
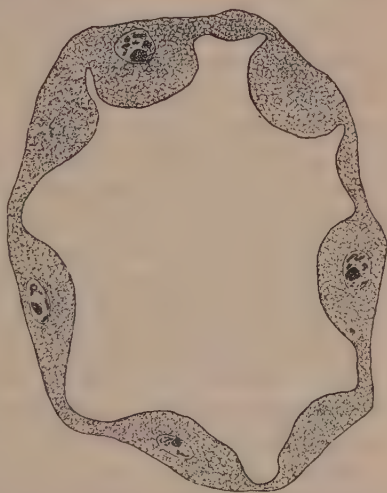

Fig. 7. Cross section through the mid-gut showing the ventral cells in the "flattened" phase.

or less extensive section of the mid-gut, waves of secretion passing over the gut, and thirdly continuous secretion over the whole surface, so that all stages may be found in one series. Probably, our material has not been quite extensive enough to solve this problem satisfactorily, but several of our preparations are strongly in favour of the second possibility. Especially one series shows an epithelium that, being rather cubical in the foremost part of the mesenteron in the middle part is extremely flat (Fig. 7), whereas in the hindmost part very high cells are to be seen, especially in the dorsal wall (Fig. 6). In this part the ventral wall, on the contrary, consists of flat cells.

Regarding the inner lining of the mesenteric epithelium, I must state that, on the whole, the information I have been able to gain seems little satisfactory. Partly, this may be due to insufficient methods but, considering the results with the hindgut, this does not seem very probable.

The trouble is in the fact that, on the one hand, in sections stained with Ehrlich's haematoxylin-eosin the cell borders show very little colour at all, while, on the other hand, in the preparations, treated with Heidenhain's haematoxylin and eosin the outermost layer of the contents shows exactly the same red colour as the cell border. The core of the content is brown in these sections.

So, even in combining the images, offered by the two staining methods, I have not been able to gain a more detailed result than this that the epithelium is lined by a border differing in colour distinctly from the rest of the protoplasm and showing a very indistinct striation. In several sections this border has been torn off from the epithelium and seems to be attached to the contents. This must be an artifact due to the fixation.

A peritrophical membrane is quite lacking.

The Proctodaeum with the Malpighian tubes

In contrast with what generally is found in larvae of Diptera, incl. Itonididae, the whole complex of the hind-gut, with the Malpighian tubes, is very voluminous compared with the mesenteron.

The proctodaeum consists of four more or less distinct divisions, which in the following will be indicated by P1, P2, P3, and P4.

The foremost division, being the largest too, at least for a hind-

gut is very thick and several times sharply curved, so that this part in its original position shows three compact loops. The compactness of these convolutions is demonstrated by Fig. 8,
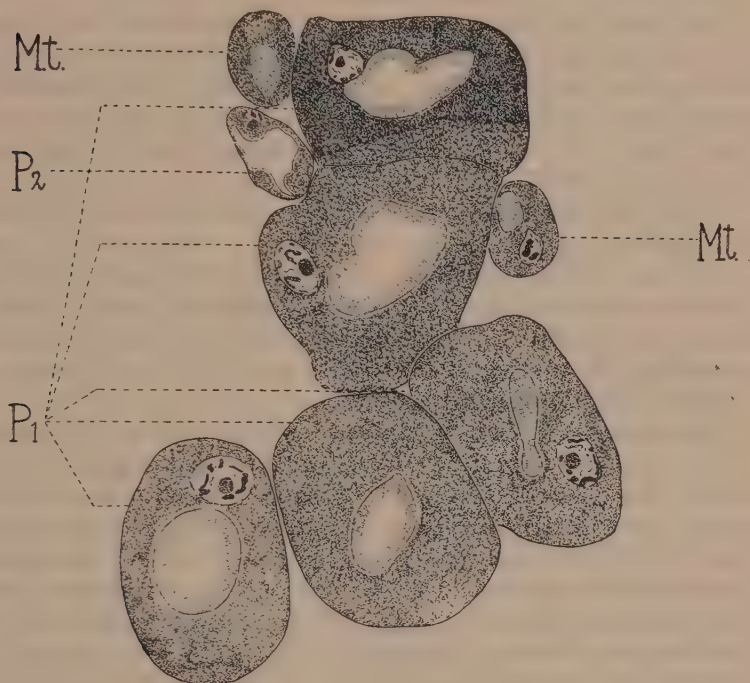

Fig. 8. Cross section showing 5 sections of P1, P2, and the Malpighian tubes. Abbreviations: M.t., Malpighian tubes; P1 and P2, parts of the proctodaeum.

showing five sections of the proctodaeum lying close against each other. In Fig. 1 the descending part of P1 is depicted some-what too much to the left in order to make the drawing not to complicated.

This part of the proctodaeum ends with an ascending loop, which a litt'e behind the level of the end of the mesenteron passes into P2.

This division is much thinner and without any loop. For some distance it runs forward, on the top of the mesenteron, but even-tually it returns and runs straight caudad. So, in all preparations

we find dorsal to the hindmost part of the mesenteron two sections of P2. In the various specimens this loop extends over a varying distance forward, probably in connection with the condition of the next division.

The third part of the hind-gut is very thin-walled and liable to expansion until this region of the body in the intact larva seems to be occupied by one large vesicle, which fills almost the whole body cavity. Into this vesicle the end of P2 passes in a similar way as the oesophagus into the mesenteron; probably this is the rectal valve of the authors.

Finally, this vesicular division is succeeded by a short tubular part ending with the anus.

The first division of the hind-gut, P1, in my opinion is by far the most remarkable part of the whole intestinal tract. As stated before, it is very thick and thick-walled (Fig. 8). Moreover, stretching the convolutions, we perceive it to be very long too, even longer than the mesenteron.

Histologically it shows a strong resemblance to the Malpigian tubes. The wall consists of an epithelium showing generally not more than three cells in one section and leaving but a small cavity. A cuticular intima is quite absent; the cavity being bordered by an even much more distinct striated membrane than that of the mesenteron. This border is strongly eosinophile and, therefore, stains very red in the specimens treated with Heidenhain's haematoxylin-eosin. The same phenomenon can be observed in the Malpighian tubes. The striated membrane passes abruptly into the cytoplasm, which all over P1 stains strongly and very evenly by haematoxylin, and in the sections stained by Heidenhain's haematoxylin seems to be finely granular. The nuclei offer no peculiarities.

Even more than in the mesenteron the wall of the gut in this part essentially is constituted by the epithelium. I have not been able to find anything like a muscle layer.

A similar specialisation of the foremost part of the hind-gut, with one exception, seems not to have been found in larvae of Diptera before. This exception regards also an Itonidid larva, i.e. *Microdiplosis reaumuri* Kieff., studied by the author of the species, who records that the foremost part of the hind-gut is just as thick as the mesenteron, from which it is separated by a constriction. Apparently, KIEFFER (1900) knew this interesting tract only from dissection, or even observation of the intact larva.

The other authors on Itonidid larvae do not mention anything like this.

To the function this part of the hind-gut is most likely to have, we will revert later on; for the moment we will put forward two conclusions which are not without interest from a morphological point of view.

First, once more we find that the presence of a cuticular intima is not such an important characteristic of the hind-gut as one is often inclined to believe.

Secondly, that the histological constitution of the Malpighian tubes hardly can be an argument for their origin from the mid-gut, the first part of the hind-gut being in our case histologically identical with the Malpighian tubes.

This thick and thick-walled part of the proctodaeum passes somewhat behind the level of the end of the mesenteron into the second division, P2. At the point of transition the epithelium becomes more flat, the cavity grows still narrower and the striated border is succeeded by a homogeneous intima, probably of a chitinous nature.

As stated above, this second segment of the proctodaeum abruptly passes into the third, the hindmost part of P2 being invaginated into P3 so as to constitute a rectal valve.

Histologically there is an enormous difference between P2 and P3. Whilst the wall of P2 is rather thick, that of P3 is extraordinarily thin, though this feature is not always clear. For, in sections of larvae, fixed with this part of the tract empty, the wall is strongly folded and, therefore, does not seem to be so

Fig. 9. Cross section through the hindmost part of the body with the circumference only indicated, showing the passing of P3 into P4.

very thin. In many larvae, on the contrary, the part in question at the time of fixation was swollen into a wide bladder (Fig. 9), visible through the body wall and in these cases the sections give the impression that this bladder lacks a proper wall. The extension just mentioned regards mainly the diameter, but the rectum is distended longitudinally, too, causing, hereby, the turning point of P2 to move forward and covering nearly the whole fourth part of the proctodaeum.

This hindmost part of the digestive tract has a much thicker wall than the foregoing division. Part of the wall, however, is not easily distinguished from the surrounding muscles of the body wall, which are pierced by it. P4 really is the only part of the proctodaeum showing clearly a chitinous intima.

The Malpighian tubes originating exactly at the spot where the mesenteron passes into the proctodaeum, are rather large and typically situated. Both the tubes immediately after their origin turn forward and for some distance run dorsal to the mesenteron. Finally they curve back, somewhat before the turning point of the proctodaeum, once again to run straightly caudad (Fig. 1).

As mentioned before, histologically the Malpighian tubes show an almost perfect resemblance to the first part of the proctodaeum. Their cavity is lined by the same striated border staining intensely with eosin and the cytoplasm shows the same granular appearance. Often in the sections the wall of a tube is constituted by one cell only, the situation of the cavity being, in this case, very eccentrical (Fig. 8).

The cavity and contents of the digestive tract

With regard to the probable way of feeding of the larvae some information about the lumen and the content of the gut is of even more importance than the knowledge of the tissues.

From the description of the fore-gut it appeared that this part of the tract is very narrow and without any dilations, which holds for the cavity too. It has also been stated that the fore-gut passes by means of a primitive cardiac valve into the wide cavity of the mesenteron, the latter in the sections being only irregularly narrowed by fixed peristaltic contractions.

One of the questions which, from the beginning, interested us most, was the presence or absence of an open passage between

the hind-gut and the mesenteron. Several older investigators as also LEEFMANS, had observed that it is impossible to remove the contents of the fresh prepared tract by pression, the mesenteron bursting before anything comes out of the anus. Since it is clear that in these cases the pression was applied to the mesenteron, the result mentioned above might be explained just as well by an occlusion of the passage between the mid-gut and the hind-gut as by an occlusion of the anus, the latter being, however, very improbable in itself.

Discontinuity of the mesenteral and proctodaeal cavities is supposed by RÜBSAAMEN and HEDICKE (1926) when saying: "Wie für andere Insekten-larven bekannt ist, scheint auch bei den Cecidomyidenlarven oder doch wenigstens bei den gallenerzeugenden, die Verbindung zwischen Mittel- und Dünndarm zu fehlen. Tatsächlich sind in vollkommen eingeschlossenen Gallen nie Spuren von Excrementen aufzufinden".

Although having at my disposal a number of complete series, it proved to be rather difficult to obtain satisfactory information about this question, owing to the fact that the foremost part of the proctodaeum is slightly invaginated into the mesenteron. Hereby and by origin of the Malpighian tubes this region of the tract is made rather indistinct. Moreover, it occupies only 2–3 sections of 5 μ. Nevertheless, what information was obtained confirmed the supposition mentioned above that a passage between mesenteron and proctodaeum is wanting. This is an important point with regard to the peculiar differentiation of the foremost division of the proctodaeum. From the description of the hind-gut sufficient information can be ontained about form and dimensions of the cavity in the several parts.

As to the contents of the digestive tract, we find the somewhat startling fact that it is only present in the mesenteron. Only in this part a homogeneous mass is to be observed, staining in a characteristic way with the different dyes, and consisting evidently of a colloid substance, coagulated during fixation. In the sections stained with Heidenhain's haematoxylin, the core of the content is brownish, whilst the layer bordering the epithelium shows the same red colour as the striated membrane of the epithelium itself. Perhaps this may be considered as an indication that the secretion of the epithelial cells diffuses but slowly into the contents.

In the contents of the mesenteron any trace of solid particles is absolutely wanting. This is one more proof for the supposition

mentioned by LEEFMANS (1937) that none but liquid food is swallowed by the larvae. Other arguments are: the reduced state of the mouth parts and the small capacity of the oesophagus.

Thus, in the sections, fore- and hind-gut are empty. That this is not so in the living larvae is obvious from the often enormous dimension of P3, this division being swollen, in such cases, into a bladder which is visible through the body wall. The complete disappearance of the contents in the sections can be explained only by the hypothesis that it merely consists of water and other substances not liable to coagulation or precipitation during fixation.

This difference between the contents of the mid-gut and the hind-gut offers additional proof that a passage between these two parts is wanting indeed.

The Malpighian tubes are also quite empty in the sections.

Which conclusions are to be drawn from the above about the way of feeding, the digestion, and the function of the procto-daeum?

First of all that the larvae of *C. torquens* feed exclusively on liquid substances, i.e. plant juices. The way in which they succeed in extracting these from the plant tissues constitutes a problem in itself. For the time being we only know that a mechanical laesion of the epidermis is out of the question. LEEFMANS, however, several times has made an experiment which yields some information. When he put a living larva on a very young dry cabbage leaf, he observed in several cases that after some time the larva was surrounded by a drop of fluid. Moreover, after removal of the larva sometimes a small depression could be seen in the surface of the leaf, corresponding with the length of the larva. In my opinion, this should be interpreted thus that, by a glandular secretion of the larva, probably the product of the salivary glands, the tissues of the plant are injured to such a degree that a certain amount of plant exsudate is secreted. Eventually, the latter is swallowed by the larva. The digestion and absorbtion of this food is limited to the mesenteron, the epithelium of which probably performs both functions continually, leaving the possibility of a rhythmical function of the individual cells.

In this chain of thought the proctodaeum has to do nothing with digestion. On the other hand, it is very improbable that

such a voluminous organ lacks any function. The nature of this function can hardly be other than excretory. Especially the structure of the foremost part of the proctodaeum with its remarkable resemblance to that of the Malpighian tubes seems to justify this hypothesis. As we have pointed out above, the larvae feed exclusively on plant juices and are, therefore, compelled to absorb quite an amount of water. Thus the presence of a voluminous excretory apparatus is easily understood. Now it is obvious too that the contents of the hind-gut consist chiefly of water. Less clear is the circumstance that it seems to be of some interest to the larva to store this water surplus in a bladder-like expansion of the gut. But perhaps this is an indication that the larvae feed intermittently, the products of the excretory organs being stored during the periods of feeding in order to prevent that the same liquid is swallowed more than one time. To gain more information about this, however, special experiments should be made.

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VERHALTENSTENDENZEN WEISSER MÄUSE IN EINEM LABYRINTH

II. RICHTUNG DER FEHLER BEI GLEICHER UND ENTGEGENGESETZTER RICHTUNG DES ZIELES UND DES LAUFES

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I. EINLEITUNG

In einer vor kurzem erschienenen Arbeit haben BIERENS DE HAAN und BIJLMER (1939), als sie das Verhalten weisser Mäuse in einer bestimmten Labyrinthsituation mit demjenigen verglichen, was bei Ratten in einer ähnlichen Situation bekannt war, gefunden, dass zwar die Mäuse im allgemeinen ähnliche Verhaltenstendenzen als Ratten aufweisen, aber doch auch bestimmte Unterschiede zwischen beiden bemerkbar sind. So fanden sie bei den Mäusen eine auch bei Ratten vorkommende Vorliebe für Sackgassen, die in der Richtung des Zieles verlaufen, sowie die Tendenz zur Antizipation der letzten Wendung und diejenige des Beibehaltens der einmal gefolgten Laufrichtung; anderseits fanden sie, dass im Vergleich zu Ratten die Tendenz zum Beibehalten der allgemeinen Laufrichtung stärker entwickelt war als die der Antizipation der letzten Wendung. Für nähere Besonderheiten sei auf die obenerwähnte Arbeit, und für die Bedeutung dieser Eigentümlichkeiten für das Verständnis des Verhaltens von Tieren in Labyrinthen auf BIERENS DE HAAN (1937) verwiesen.

Uns fiel nun besonders der letztgenannte Unterschied auf: Mäuse scheinen mehr als Ratten dazu geneigt zu sein, die allgemeine Laufrichtung beizubehalten. Die Frage war nun, ob dies eine Eigentümlichkeit der verwendeten Tiere war oder mit der benutzten Labyrinthsituation zusammenhing, oder ob wir dies als einen mehr allgemeinen Unterschied zwischen Ratten und Mäusen betrachten dürfen.

Um dies näher zu prüfen, schien ein Labyrinth sehr geeignet, das von DASHIELL (1930) bei Ratten benutzt worden war, und das mit dem in Abb. 1 abgebildeten im Prinzip überein-

stimmt. Wie man in der Abbildung sieht, besitzt dieses Laby-
rinth 8 Sackgassen, die in vier Paaren von gleicher Form und
Grösse angeordnet sind. In diesen Paaren erstreckt sich je eine
Sackgasse in die Vorwärts- und eine in die Rückwärts-Richtung,
gerechnet nach der allgemeinen Laufrichtung (in der Figur be-
zeichnet mit V_1–V_4, bezw. R_1–R_4). Am Ende des eigentlichen
Labyrinthes befinden sich zwei Klappen K_v und K_r, die wegge-
nommen oder angebracht werden können; ist K_r eingesteckt, dann
steht dem Tier nur der Weg zum vorderen Futternapf F_v frei, ist K_v
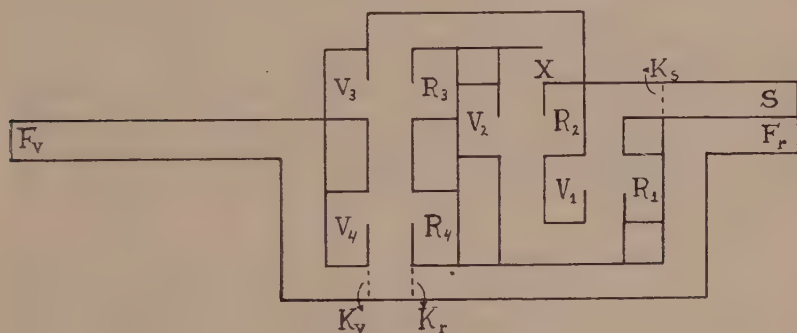
Abb. 1

eingesteckt so kann das Tier nur zum hinteren Futternapf F_r laufen.
Es zeigte sich DASHIELL nun, dass auf den vorderen Futternapf
dressierte Ratten mehr Fehler in den vorwärts gerichteten, den
V-Sackgassen, als in den rückwärts gerichteten R-Sackgassen
machten (in einer Serie 74,4% gegen 25,6%; in zwei anderen Serien
66% gegen 34 % und 64,1% gegen 35,9%), während dagegen
Ratten, die ihren Futternapf an der rückwärts liegenden Seite
des Labyrinthes fanden, mehr Fehler in der rückwärtigen
Richtung machten und zwar bezw. 52,4% gegen 47,6,%, 53,5%
gegen 46,5% und 54,9% gegen 45,1% in die rückwärts, bezw.
vorwärts liegenden Gassen hineinliefen. Es zeigt sich hieraus
also, dass die Ratten mehr Fehler in Sackgassen machten,
die *in der Richtung ihres Zieles* lagen als in Sackgassen in der
entgegengesetzten Richtung. Aber auch zeigte es sich, wenn man
DASHIELLS Zahlen vergleicht, dass diese Unterschiede mehr aus-
gesprochen waren bei Tieren, die auf ein Ziel in der vorwärts
liegenden als bei solchen, die auf eins in der rückwärts liegenden
Richtung dressiert waren. Die Erklärung dieser Unterschiede

lag auf der Hand: es wirkten hier zwei Tendenzen zusammen, bezw. einander entgegen, nämlich die Tendenz, in Sackgassen *in der Richtung des Zieles* und die Tendenz, in Sackgassen *in der Richtung der allgemeinen Laufrichtung* hineinzulaufen. Bei den auf ,,vorwärts'' dressierten Tieren wirkten diese Tendenzen zusammen; bei den auf ,,rückwärts'' dressierten Tieren hoben sie einander teilweise auf. Und da wir gerade diese beiden Tendenzen bei unseren Mäusen gegen einander abwägen wollten, schien uns DASHIELLS Apparat zur Beantwortung unserer Frage sehr geeignet.

Der von uns benutzte Apparat (Abb. 1) war nach dem Prinzip des DASHIELLschen aufgebaut und den Körperdimensionen der Mäuse angepasst. Die Höhe der Holzwände war 10 cm, die Breite der Laufgänge 5 cm. Der Weg vom Startpunkt S bis zu K_v und K_r war ungefähr 150 cm; die Wege von K_v zum vorwärts stehenden Futternapf F_v und von K_r zum Futternapf F_r waren beide gleich lang und etwa 70 cm. Es war dafür gesorgt, dass die Tiere in beiden Richtungen nach dem Eintreten in den Endgang noch eine gleiche Zahl (zwei) Wendungen machen mussten, bevor sie das Ziel erreichten.

Als Versuchstiere verwandten wir wiederum junge männliche weisse Mäuse, die uns aus einer Mäusezucht des Amsterdamer Instituts für Krebsforschung zur Verfügung gestellt worden waren. Beim Anfang der Versuche waren die Tiere etwa 4 Monate alt. Sie wurden in zwei Gruppen von je 9 Tieren eingeteilt; in jeder Gruppe wurden die Tiere zur individuellen Unterscheidung am Kopf und Rücken mit Merkzeichen versehen, und zwar bei der einen (sog. roten) Gruppe mit Karbolfuchsin, bei der anderen (sog. blauen) Gruppe mit Karbolgentianviolett. Jeden Tag wurde mit jedem Tier nur ein Versuch angestellt; abwechselnd wurde an jedem Versuchstage entweder zuerst mit der roten oder mit der blauen Gruppe gearbeitet. Beim Anfang eines jeden Versuches wurden die Tiere beim Startpunkt S in das Labyrinth hineingesetzt; und dann wurde nach einigen Augenblicken die Klappe K_s weggenommen, wodurch der Weg frei kam. Anfangs wurde die blaue Gruppe auf den in der Vorwärtsrichtung, die rote auf den in der Rückwärtsrichtung stehenden Futternapf dressiert. In diesem Futternapf fanden die Tiere, die an demselben Tag nog nicht gefressen hatten, Vogelsamen, Hanf und Milch. Die Hauptfütterung fand jedesmal nach den Versuchen statt. Als Fehler galt es, wenn die Tiere die Nase in eine Sackgasse steckten, oder wenn sie auf dem Hauptweg zurückliefen. Ausserdem wurden die Laufzeiten mit der Stoppuhr bestimmt. Als Laufzeit galt die Zeit, die zwischen dem Augenblick, in welchem die Klappe beim Startpunkt weggenommen wurde, bis zu dem Momente, wenn die Maus das Futter berührte, verlief. Wie früher (BIERENS DE HAAN, 1937) schon betont wurde, hat diese Zeit aber nur einen untergeordneten Wert. Am ersten Versuchstage wurde den Tieren Gelegenheit gegeben, den Apparat und die Belohnung am Ziele im allgemeinen kennen zu lernen; Fehler und Zeiten wurden an diesem Tage nicht verzeichnet. Danach fingen die eigentlichen Versuche an.

II. DRESSUR DER BLAUEN GRUPPE AUF DEN IN DER VOR-WÄRTS-RICHTUNG STEHENDEN FUTTERNAPF F_v UND DER ROTEN AUF DEN IN DER RÜCKWÄRTS RICHTUNG STEHENDEN FUTTERNAPF F_r.

Wie gesagt, wurde zuerst die blaue Gruppe auf den vorwärts, die rote auf den rückwärts stehenden Futternapf dressiert.

Die Dressur der blauen Gruppe verlief sehr flott; das Laby-rinth war offenbar für die Tiere leicht zu erlernen. Am schnell-sten gelang die Dressur bei dem Tiere Blau 2, das am ersten Tage nur einen Fehler machte (in V_3) und dann vom 2.—6. Tage überhaupt fehlerlos das Labyrinth durchlief. Am 7. Versuchstage machte dieses Tier aber plötzlich wieder 10 Fehler und am 11. Tage 12, aber nach diesem Tage wurden keine Fehler mehr gemacht. Das Tier Blau 9 machte nach dem 3. Versuchstage nur noch einmal 2 Fehler, und zwar am 12. Tage. Bei den anderen Tieren wechselten fehlerfreie Tage mit Tagen mit Fehlern ab; nach dem 12. Tag wurden jedoch praktisch keine Fehler mehr gemacht, sodass am 15. Tage das von uns als Kriterium des Lernens gestellte: ,,Die ganze Gruppe drei Tage fehlerlos'' erreicht wurde. (Eigentlich wurde am 15. Tage noch ein Fehler gemacht, und zwar vom Tiere Blau 5; dies geschah, als während des Laufens plötzlich die Zimmertür geöffnet wurde und da das Tier die beiden vorigen Tage fehlerlos gelaufen hatte, wollten wir es ihm nicht als einen ernsten Fehler anrechnen). Die Laufzeiten nahmen während der Versuche natürlich stark ab, und betrugen am Ende nur noch 6–8 Sekunden.

Sowohl bei dieser als bei den anderen Dressuren traten bestimmte Typen von Verirrungen hervor. So sahen wir oft, dass Tiere, die das Labyrinth schon einigermassen kannten, wenn sie irgendwo in der Mitte des Apparates einen Fehler gemacht hatten, ganz zum Startpunkt zurückliefen und erst von dort aus wieder fehlerlos das Labyrinth durchliefen. Auch BIERENS DE HAAN und BIJLMER hatten dies bei ihren Tieren beobachtet. Dies spricht dafür, dass die Tiere sich gewisser-massen eine Vorstellung vom Labyrinthwege gebildet haben, die aber gestört wird, wenn sie aus irgendeinem Grunde in eine fehlerhafte Sackgasse hineingelaufen sind. Das Tier flüchtet dann zum Startpunkt zurück, von wo aus der Weg wieder bekannt ist. Auch sahen wir oft, dass, wenn ein Tier einen Fehler in einer der Sackgassen mit hohem Index gemacht hatte,

es zurücklief und dann anfing, Fehler in den ersten Sackgassen zu machen. In solchen Fällen war das Bild des Weges wesentlicher gestört, und fing das Tier an, an Stellen zu stümpern, wo es vorher richtig den guten Weg gekannt hatte. Auch kam es oft vor, dass, wenn ein Tier nach einem Fehler aus einer der V- oder R-Sackgassen hinauslief, es sich in die gegenüberliegende Gasse begab. Dann war es, als ob das Tier, das an dieser Stelle geradeaus hätte laufen müssen, jetzt die gegenüberliegende Sackgasse für den geraden Weg hielt. Und schliesslich ergaben sich am Anfange häufig Schwierigkeiten an der Stelle, wo bei X in der Abbildung eine Drehung von 180° gemacht werden musste. Hier zögerten die Tiere dann und liefen auf ihrem Weg zurück, ohne vorher in einer Sackgasse festgelaufen zu sein. Da natürlich keine mechanischen Schwierigkeiten für eine solche Drehung vorlagen, müssen wir annehmen, dass eine Drehung um 180° sich etwas schwierig in das Bild des Weges einschalten lässt.

Was nun die Verteilung der Fehler auf die verschiedenen Sackgassen betrifft, sehe man Tabelle I, wo man die Fehler-

TABELLE I. Fehlerverteilung bei der Dressur der blauen Gruppe auf den vorwärtigen Futternapf F_v.

Ver-suchs-tag	rückwärts					vorwärts					zurück	zus.
	R_1	R_2	R_3	R_4	zus.	V_1	V_2	V_3	V_4	zus.		
I	17	4	16	8	45	16	3	12	12	43	33	121
II	3	6	2	7	18	4	8	4	8	24	13	55
III	5	7	3	7	22	6	5	7	6	24	13	59
IV	6	4	2	7	19	4	3	1	8	16	7	42
V	4	0	1	4	9	4	2	2	8	16	11	36
VI	2	1	1	1	5	6	5	2	5	18	14	37
VII	1	1	0	1	3	2	5	0	4	11	6	20
VIII	1	2	0	1	4	2	8	3	5	18	9	31
IX	4	0	2	7	13	10	6	3	12	31	10	54
X	2	2	1	5	10	11	4	1	11	27	19	56
XI	5	0	0	2	7	10	2	0	4	16	9	32
XII	2	1	0	0	3	8	0	0	1	9	13	25
XIII	0	0	0	0	0	0	0	0	0	0	0	0
XIV	0	0	0	0	0	0	0	0	0	0	0	0
XV	0	0	0	0	0	0	(1)	0	0	(1)	0	0
Zus.	52	28	28	50	158	83	52	35	84	254	157	569
Proz.	12,6	6,8	6,8	12,1	38,3	20,1	12,6	8,5	20,4	61,6	—	99,9

verteilung der gesamten Gruppe an jedem Tage, sowie die Gesamtzahlen und die prozentuale Verteilung der Fehler auf die verschiedenen Sackgassen verzeichnet findet. Besonders die letztgenannte Verteilung ist sehr lehrreich. Wie man sieht, wurde im ganzen 61,6% der Fehler in den vorwärts liegenden gegenüber 38,3% in den rückwärts liegenden Sackgassen gemacht, eine Zahl, die ungefähr mit den DASHIELLschen Zahlen bei Ratten übereinstimmt. In beiden Gruppen fielen etwa 2/3 der Fehler auf die Sackgassen 1 und 4, während in die beiden mittleren Sackgassen weniger hineingelaufen wurde. Die Fehler in den ersten Sackgassen sind wohl teilweise durch die Unsicherheit verursacht, die das Tier im Anfange des Apparates zeigt; die Fehler am Ende sind teilweise der Tendenz zur Antizipation zuzuschreiben, die das Tier verleitet, zu früh   in die 4. vordere Sackgasse abzubiegen, wobei die Tiere oft, wie wir sahen, wenn sie sich in die 4. vordere Sackgasse verirrt hatten, nachher noch in die gegenüberliegende Sackgasse hineinliefen. Überzeugend zeigt sich vor allem: *grössere Vorliebe für die in der Vorwärtsrichtung liegenden Sackgassen bei vorwärts liegendem Ziel.*

Zur gleichen Zeit, als wir die blaue Gruppe auf den in der Vorwärtsrichtung befindlichen Futternapf dressierten, wurde die rote Gruppe auf den rückwärts stehenden Napf dressiert. Diese Dressur verlief ganz anders als die Dressur in Vorwärtsrichtung. Es zeigte sich, dass die Tiere dasselbe Labyrinth mit dem Ziel an der Rückseite überhaupt nicht fehlerfrei erlernen konnten! Jedenfalls hatte in 30 Tagen, also in der doppelten Zeit, welche die andere Gruppe für die Vorwärtsdressur benötigt hatte, die Gruppe als Ganzes noch nicht einen einzigen fehlerlosen Tag gehabt, obwohl sie am 21., 22. und 27.–29. Tage diesem Ideal sehr nahe kam, und auch alle Tiere individuell wohl fehlerfreie Tage gehabt hatten. Am besten arbeitete wohl das Tier Rot 1, das am 2. Versuchstage ganz fehlerfrei lief, aber dann wieder anfing, Fehler zu machen und erst vom 17. Versuchstage an keine Fehler mehr machte, und das Tier Rot 2, das am 5. Tage seinen ersten fehlerfreien Gang lief und nach dem 16. Tage keine Fehler mehr machte. Dagegen dauerte bei den Tieren Rot 6 und Rot 7 der Zustand an, dass fortwährend Tage mit und ohne Fehlern einander abwechselten. Offenbar war das Labyrinth mit rückwärts liegendem Ziele viel schwieriger zu erlernen als mit vorwärts liegendem Ziel.

TABELLE II.   Fehlerverteilung bei der Dressur der roten Gruppe auf den rückwärtigen Futternapf F_r.

Versuchs-tag	rückwärts					vorwärts					zurück	zus.
	R_1	R_2	R_3	R_4	zus.	V_1	V_2	V_3	V_4	zus.		
I	41	15	11	22	89	39	18	14	21	92	62	243
II	14	19	20	11	64	15	14	4	10	43	23	130
III	4	7	13	20	44	4	7	8	17	36	22	102
IV	10	6	6	9	31	13	14	6	12	45	21	97
V	2	0	0	1	3	6	4	0	2	12	8	23
VI	3	4	3	4	14	9	6	2	5	22	12	48
VII	10	2	0	1	13	13	7	1	0	21	12	46
VIII	0	1	0	1	2	7	3	1	1	12	3	17
IX	2	1	1	2	6	9	1	1	1	12	7	25
X	1	1	0	0	2	7	7	0	0	14	6	22
XI	3	2	0	1	6	6	3	0	1	10	6	22
XII	0	1	0	1	2	7	3	0	2	12	7	21
XIII	1	1	1	3	6	1	2	1	4	8	3	17
XIV	0	0	0	1	1	3	0	0	0	3	1	5
XV	1	1	0	0	2	7	1	0	0	8	6	16
XVI	0	0	0	1	1	2	0	0	0	2	3	6
XVII	1	0	0	6	7	1	1	1	2	5	4	16
XVIII	1	0	0	7	8	0	0	2	3	5	5	18
XIX	0	1	0	1	2	1	1	0	0	2	2	6
XX	0	0	0	1	1	5	0	0	0	5	4	10
XXI	0	0	0	0	0	1	0	0	0	1	1	2
XXII	0	0	0	0	0	2	0	0	0	2	1	3
XXIII	3	1	0	1	5	3	2	0	1	6	5	16
XXIV	4	0	0	0	4	4	0	0	0	4	3	11
XXV	4	3	0	0	7	5	2	1	2	10	7	24
XXVI	0	0	0	0	0	1	1	0	1	3	2	5
XXVII	0	0	0	0	0	0	1	0	0	1	2	3
XXVIII	0	0	0	1	1	0	0	0	1	1	1	3
XXIX	0	0	0	0	0	1	0	0	1	2	1	3
XXX	1	0	0	1	2	3	0	0	0	3	4	9
Zus.	106	66	55	96	323	175	98	42	87	402	244	969
Proz.	14,6	9,2	7,6	13,2	44,6	24,1	13,5	5,6	12,0	55,2	—	99,8

In Tabelle II geben wir die gesamtzahle der 30 Versuchstage und auch wieder die prozentuale Verteilung der Fehler auf die verschiedenen Sackgassen wieder. Die Vergleichung mit der Fehlerverteilung der blauen Gruppe ist interessant. Während, wie wir sahen, die blauen Mäuse 61,6% der Fehler in denjenigen Sackgassen machten, die vorwärts (also in die Richtung des Zieles) gerichtet waren, gegenüber 38,3% in den rückwärts

gerichteten Sackgassen, machten die roten Tiere 55,2% ihrer Fehler in den vorwärts gerichteten Sackgassen, die jetzt vom Ziele wegführten, gegenüber 44,6% in den rückwärts gerichteten Sackgassen, die jetzt zum Ziele hinwiesen. Im Vergleich zu den vorwärts dressierten blauen Tieren machten die roten also etwa 6,4% mehr Fehler in den rückwärts gerichteten und 6,4% weniger in den vorwärts gerichteten Sackgassen. Dies zeigt wieder den Einfluss der Richtung des Zieles: *das rückwärts liegende Ziel verlockt zu Fehlern in den rückwärts gerichteten Sackgassen.* Aber wenn wir diese Zahlen mit den Ergebnissen DASHIELLS vergleichen, finden wir doch wieder einen Unterschied zwischen Ratten und Mäusen. Wenn wir die Mittelwerte der drei DASHIELLschen Versuchsserien nehmen, sehen wir, das seine vorwärts dressierten Ratten 68,1% Fehler in der Richting nach vorn und 31,8% in rückwärtiger Richtung machten, seine rückwärts dressierten dagegen 46,4% in den vorwärts und 53,6% in den rückwärts gerichteten Sackgassen. Hier waren die Prozente also um 21,7% gestiegen, bezw. gefallen, ein Unterschied, der etwa 3,5 mal grösser ist als bei unseren Mäusen! Die Erklärung dieses Unterschieds muss sein, dass, wie dies BIERENS DE HAAN und BIJLMER schon gefunden hatten, die Tendenz zum Beibehalten der allgemeinen Laufrichtung bei Mäusen stärker entwickelt ist als andere, den Lauf im Labyrinth mitbestimmende Tendenzen, wie die der Richtung auf das Ziel. Dies zeigt sich auch noch daraus, dass wir im Gegensatz zu DASHIELL bei seinen Ratten bei unseren Mäusen nicht fanden, dass die grössere Hälfte der Fehler (53,6%) in einer Richtung gemacht wurde, die gegen die allgemeine Laufrichtung verlief. Die am Anfang gestellte Frage muss also in dem Sinne beantwortet werden, dass wirklich die grosse Entwicklung dieser Tendenz eine allgemeine Eigenschaft weisser Mäuse zu sein scheint. Die grössere Schwierigkeit des Labyrinthes mit rückwärts gerichteten Ziele wird hiermit wohl zusammenhängen: Beim Labyrinth mit rückwärtigem Ziele ist die allgemeine Laufrichtung der zweiten Hälfte der Bahn (nach dem Punkte K_v) derjenigen der ersten Hälfte entgegengesetzt, und da die Tiere diese Laufrichtung relativ so stark anzufühlen scheinen, wird die Einheitlichkeit des Laufens durch diesen Gegensatz gestört und das Erlernen erschwert.

Wenn wir auch bei dieser Dressur auf die relative Verteilung der Fehler auf die verschiedenen Sackgassen in beiden Richtun-

gen achten, so sehen wir, dass bei den Sackgassen beider Art wieder etwa 2/3 der Fehler auf die Sackgassen 1 und 4 entfielen. Besonders hoch war jetzt die Zahl der Fehler in V_1, in welcher fast ein Viertel aller Fehler gemacht wurde. Eine befriedigende Erklärung hiervon ist nicht gut zu geben.

III. UMDRESSUR DER BLAUEN GRUPPE AUF RÜCKWÄRTS UND DER ROTEN AUF VORWÄRTS

Im Anschluss an die obigen Dressuren wollten wir dann noch untersuchen, was geschieht, wenn man Tiere, die erlernt haben das Ziel zu erreichen, das vor oder hinter dem eigentlichen Labyrinth liegt, jetzt dasselbe Labyrinth durchlaufen lässt, während der Futternapf in entgegengesetzter Richtung wie vorher steht. Die Frage war dann, ob bei dieser Umstellung des Zieles von vorwärts nach rückwärts oder umgekehrt Fehler beim Durchlaufen des unveränderten, schon erlernten eigentlichen Labyrinths auftreten, und ob in diesen Fehlern wieder etwas von den oben studierten Tendenzen zu beobachten ist.

Zuerst wurde dazu bei der blauen Gruppe, die, wie wir sahen, in 15 Tagen erlernt hatte, das Labyrinth mit vorn liegenden Ziel zu durchlaufen, die Klappe K_r weggenommen und die Klappe K_v eingesetzt, sodass die Tiere zu dem rückwärtigen Futternapf laufen mussten. Wie zu erwarten war, liefen die Tiere am ersten Tag der neuen Dressur, der sich der vorigen Dressur direkt anschloss, ohne Fehler zum Scheidepunkt K. Wider Erwarten waren sie dann aber garnicht überrascht. als sie jetzt nach links statt nach rechts abbiegen mussten: sie liefen alle ohne Fehler zum rückwärtigen Futternapf. Höchstens konnte man bemerken, dass sie etwas zögernd und vorsichtig das neue Endstück liefen, nachdem sie den altbekannten eigentlichen Labyrinthweg schnell und resolut durchlaufen hatten. Während die Mäuse am letzten Tage der vorigen Dressur durchschnittlich in 7,4 Sek. den Futternapf erreichten, brauchten sie dafür jetzt durchschnittlich 11,3 Sek.; wenn man dabei bedenkt, dass der Weg durch das Labyrinth etwas mehr als zweimal länger war als das Endstück, kann man sagen, dass das neue Endstück etwa $2\frac{1}{2}$ mal langsamer durchlaufen wurde als das gleich lange alte Endstück. Am zweiten Versuchstage machte ein Tier (No. 5) 9 Fehler im Labyrinth und ein anderes 2; abgesehen hiervon wurden in den ersten 5 Tagen der neuen Dressur keine Fehler gemacht.

Später traten jedoch bei mehreren Tieren Fehler auf. Doch gab es drei Tiere (No. 4, 8 und 9), die von den 25 Tagen, während welcher die neue Dressur fortgesetzt wurde, 24 mal fehlerlos liefen und nur einmal 1 bis 3 Fehler machten. Am schlechtesten war No. 5 mit seinen 9 Fehlern am 2. Tage und später noch einmal 4 Fehlern, und No. 7, das einmal 8 Fehler machte. Man kann, alles zusammengenommen, also nicht sagen, dass die Tiere durch die Umstellung des Zieles ausserhalb des eigentlichen Labyrinthes vom Wege im Labyrinth abgebracht wurden.

TABELLE III. Fehlerverteilung bei der Umdressur der blauen Gruppe auf den rückwärtigen Futternapf F_r.

Ver-suchs-tag	rückwärts					vorwärts					zurück	zus.
	R_1	R_2	R_3	R_4	zus.	V_1	V_2	V_3	V_4	zus.		
I	0	0	0	0	0	0	0	0	0	0	0	0
II	0	2	0	1	3	1	2	0	1	4	4	11
III	0	0	0	0	0	0	0	0	0	0	0	0
IV	0	0	0	0	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0	0	0	0	0
VI	0	0	0	5	5	0	0	0	2	2	2	9
VII	0	0	2	2	4	0	0	1	1	2	1	7
VIII	0	0	0	1	1	0	0	0	0	0	1	2
IX	0	2	0	1	3	0	0	0	0	0	1	4
X	0	1	0	1	2	0	0	0	0	0	0	2
XI	0	1	0	0	1	0	0	0	0	0	0	1
XII	0	0	0	0	0	0	0	0	0	0	0	0
XIII	0	0	0	0	0	0	0	0	0	0	2	2
XIV	2	2	0	0	4	0	2	0	0	2	3	9
XV	0	0	0	1	1	0	0	0	0	0	0	1
XVI	0	0	0	0	0	2	0	0	0	2	3	5
XVII	3	0	0	0	3	5	0	0	0	5	4	12
XVIII	0	0	0	0	0	1	0	0	0	1	1	2
XIX	1	1	0	0	2	3	0	0	0	3	3	8
XX	0	0	0	0	0	0	0	0	0	0	0	0
XXI	0	1	0	0	1	0	0	0	0	0	1	2
XXII	0	1	0	0	1	0	1	0	0	1	1	3
XXIII	0	0	0	0	0	0	0	0	0	0	0	0
XXIV	0	0	0	0	0	1	0	0	0	1	0	1
XXV	0	0	0	1	1	0	0	0	0	0	0	1
Zus.	6	11	2	13	32	13	5	1	4	23	27	82
Proz.	10,9	20,0	3,6	23,6	58,1	23,6	9,1	1,8	7,2	41,7	—	99,8

Doch wurden in den 25 Tagen zusammen 82 Fehler, wovon 55 in den Sackgassen, gemacht. Was lehrt uns nun die Verteilung dieser Fehler? Dafür sehe man Tabelle III.

Während bei der ersten Dressur, wie wir sahen, 61,6% der Fehler in den vorwärts gerichteten gegenüber 38,3% in den rückwärts gerichteten Sackgassen gemacht wurden, wurden jetzt 41,7% in den vorwärts und 58,1% in den rückwärts gerichteten gemacht. Abgesehen von einem Unterschied von 3,5% waren die Zahlen also jetzt gerade umgekehrt wie vorher. *Das jetzt rückwärts liegende Ziel zog also gleich stark an als damals das vorwärts liegende.* Nur die Verteilung der Fehler je auf die vorwärts, bezw. rückwärts gerichteten Sackgassen war anders als vorher und weniger regelmässig. Bei den vorwärts gerichteten Sackgassen zog vornehmlich V_1 und bei den rückwärts gerichteten R_4 stark an, sodass in diesen beiden zusammen fast die Hälfte aller Fehler gemacht wurde. Bei der grossen Zahl der Fehler in R_4 wird wohl wieder die Antizipationstendenz ihre Rolle gespielt haben und bei der Zahl in V_1 wieder die Unsicherheit beim Eintritt in das Labyrinth; unerklärbar bleibt dann aber die auch ziemlich grosse Fehlerzahl in R_2. Aber es kann nicht geleugnet werden, dass die relativ geringe Fehlerzahl zu viel vom Zufall mitbestimmt wurde und dadurch keine Erklärung gestattet.

Es fällt aber bei Vergleichung der Fehlerzahlen noch etwas anderes auf. Als wir, sei es auch nicht ganz erfolgreich, die rote Gruppe auf den rückwärts stehenden Futternapf dressieren wollten, wurde, wie wir sahen, die Mehrheit der Fehler (55,2%) in den vorwärts gerichteten Sackgassen gemacht. Wir sahen darin eine Anweisung dafür, dass die Tendenz zum Beibehalten der allgemeinen Laufrichtung stärker entwickelt war als die Tendenz der Richtung auf das Ziel. Jetzt, als wir die einmal dressierten blauen Mäuse auf das rückwärtige Ziel umdressierten, wurden die meisten Fehler (58,1%) in den rückwärts gerichteten Sackgassen gemacht. Die Ursache dieses Unterschiedes muss in der Weise der Dressur liegen. Die roten Tiere mussten durch das Labyrinth hindurch den Weg zu dem rückwärtigen Ziel erlernen; die blauen lernten, als das eigentliche Labyrinth schon bekannt war, am Ende zurückzubiegen. Offenbar war bei Rot das Streben, den Weg durch das Labyrinth zu finden, leitend bei der Bestimmung der Fehler, während die blauen Tiere, die diesen Weg schon kannten, nicht mehr bestrebt

waren, das Labyrinth zu erlernen, wodurch das Schlusstück des ganzen Weges (die Strecke von K_v zum Futternapf F_r) einen stärkeren Einfluss ausüben und die Richtung der Fehler beherrschen konnte.

TABELLE IV. Fehlerverteilung bei der Umdressur der roten Gruppe auf den vorwärtigen Futternapf F_v.

Ver-suchs-tag	rückwärts					vorwärts					zurück	zus.
	R_1	R_2	R_3	R_4	zus.	V_1	V_2	V_3	V_4	zus.		
I	1	2	1	1	5	1	2	0	0	3	4	12
II	0	1	0	0	1	3	0	0	1	4	2	7
III	1	1	0	0	2	7	0	0	0	7	5	14
IV	1	1	0	0	2	3	1	0	3	7	4	13
V	1	0	0	0	1	1	0	0	4	5	4	10
VI	0	0	0	0	0	1	0	0	2	3	1	4
VII	0	0	0	0	0	0	0	0	2	2	1	3
VIII	1	1	0	0	2	3	0	0	4	7	7	16
IX	0	1	0	1	2	2	1	0	2	5	6	13
X	1	0	0	0	1	2	0	0	2	4	1	6
XI	1	0	0	0	1	1	0	0	3	4	4	9
XII	0	2	0	0	2	0	1	1	1	3	2	7
XIII	0	0	0	0	0	1	0	0	1	2	2	4
XIV	1	0	0	0	1	1	0	0	3	4	2	7
XV	1	1	0	0	2	1	1	0	1	3	1	6
XVI	2	2	0	0	4	1	0	0	0	1	2	7
XVII	0	2	1	1	4	0	2	0	2	4	5	13
XVIII	2	2	0	0	4	1	1	1	0	3	2	9
Zus.	13	16	2	3	34	29	9	2	31	71	55	160
Proz.	12,4	15,2	1,9	2,9	32,4	27,6	8,5	1,9	29,5	67,5	—	99,9

Schliesslich haben wir auch die rote Gruppe, die, wenn auch nicht vollkommen, auf den rückwärtigen Futternapf dressiert war, auf den vorderen umdressiert. Tabelle IV bringt die Fehlerverteilung für die ganze Gruppe. Wie man sieht, waren die Zahlen anfangs etwas schlechter als am Ende der gerade vorhergehenden rückwärtigen Dressur, aber bald wurde wieder eine ungefähr ähnliche Fehlerzahl erreicht. Die Durchlaufzeiten waren anfangs auch etwas grösser: durchschnittlich 12 Sek. am ersten Tag der neuen Dressur gegenüber 9,5 Sek. am letzten Tage der ersten. Die Dressur konnte nur 18 Tage fortgesetzt werden. In dieser Zeit, die länger als diejenige ist, welche die

blaue Gruppe benötigt hatte, um den Weg zum vorderen Futternapf fehlerfrei zu erlernen, wurde dieser Weg gegen unsere Erwartung nicht von der roten Gruppe erlernt. Offenbar hatte die zu schwierige Dressur auf den rückwärtigen Futternapf die Tiere so verwirrt, dass sie jetzt auch nicht mehr den einfacheren Weg zum vorderen Futternapf zu lernen imstande waren. Natürlich machten die Tiere individuell wohl fehlerfreie Läufe: Das Tier Rot 1, das bei der ersten Dressur zu den besten gehörte, machte in den 18 Tagen 14 fehlerlose Gänge, darunter eine Serie von 10 aufeinanderfolgenden, und Rot 2 machte sogar nur am ersten Tage 2 Fehler, und lief dann weiter ganz fehlerfrei. Rot 6 war jetzt besser als vorher, und machte in den 10 letzten Tagen keine Fehler mehr; am schlechtesten war diesmal Rot 9, welches nur 2 mal fehlerfrei lief. Aber die Gruppe als Ganzes lernte den Weg nicht.

Wenn wir dann wieder die Fehlerverteilung dieser Gruppe betrachten, zeigt es sich aus Tabelle IV, dass jetzt wieder die grosse Mehrheit der Fehler (67,5% gegen 32,4%) in den vorwärts gerichteten Sackgassen gemacht wurde. Die Unterschiede waren jetzt noch grösser als bei der ersten Dressur der blauen Gruppe (61,6% gegen 38,3%), und natürlich viel grösser, als wenn dieselbe Gruppe auf den rückwärtigen Futternapf dressiert wurde. Bei den rückwärtigen Sackgassen zogen jetzt vor allem die beiden ersten an; bei den vorwärts gerichteten waren es hauptsächlich V_1 und V_4. Wenn wir in dem letzten wieder den Einfluss der Antizipationstendenz erkennen wollen, so bleiben die grösseren Zahlen in den anderen Sackgassen eigentlich unerklärt.

IV. VERSUCH EINER NÄHEREN ANALYSE DES LERNENS

Zum Schlusse haben wir uns noch gefragt, ob sich vielleicht noch etwas mehr über das Lernen selbst sagen liesse, und ob vielleicht die verschiedenen Tendenzen, die wir oben besprachen, nacheinander zutagetreten, und dadurch verschiedene Perioden beim Lernen markieren würden. Obwohl die Zahl der Tiere, und damit auch die Zahl der Fehler, besonders bei dem Unlernen, ziemlich klein ist, zeigt es sich doch, dass bei jeder Dressur, bezw. Umdressur drei aufeinanderfolgende Perioden zu unterscheiden sind. Die *erste Periode*, die nur wenige Tage dauert, ist durch eine allgemeine Unsicherheit in der neuen Aufgabe gekennzeichnet, welche Unsicherheit sich darin

äussert, dass, möge die Zahl der Fehler gross sein (wie bei der
ersten Dressur) oder klein (wie bei der Umdressur), die Fehler-
zahlen in Vorwärts- und Rückwärtsrichtung ungefähr gleich
sind. Dann folgt eine *zweite Periode*, die etwa 10 Tage dauert.
In dieser Zeit fallen bei der ersten Dressur die Fehlerzahlen täglich
deutlich herunter, und es werden nicht mehr etwa gleiche Zahlen
Fehler in beiden Richtungen gemacht, sondern die Orientierung
zum Ziele macht ihren Einfluss bemerkbar, sodass mehr Fehler
in Vorwärtsrichtung gemacht werden, wenn das Ziel vorwärts
liegt und mehr in rückwärtiger Richtung, wenn es rückwärts
liegt. Eine Ausnahme macht nur die erste Dressur von Rot
auf den rückwärtigen Futternapf, worauf wir schon oben (S. 386)
hinwiesen. Schliesslich folgt dann eine *dritte Periode*, in welcher
die Fehlerzahlen nochmals stark fallen, und in bestem Falle
sich dem Werte Null nähern. In dieser Periode finden wir bei
allen vier Dressuren ein, wenn auch natürlich nur geringes

TABELLE V.   Die drei Perioden des Lernens.

Tage	Fehler in rückwärtiger Richtung	Fehler in vorwärtiger Richtung
A. Blau, erste Dressur, Futter vorwärts		
1—4	104	107
5—12	54	146
13—15	0	1
B. Blau, zweite Dressur, Futter rückwärts		
1—5	3	4
6—15	21	6
16—25	8	13
C. Rot, erste Dressur, Futter rückwärts		
1—4	228	216
5—16	58	136
17—30	37	50
D. Rot, zweite Dressur, Futter vorwärts		
1—2	6	7
3—12	13	47
13—18	15	17

Überwiegen der Fehler in der Richtung des Laufes, also vor-
wärts. Das Labyrinth ist jetzt bekannt, die Vorwärtstendenz
bleibt wirken. In Tabelle V findet man diese Einteilung durch
Zahlen bestätigt, die, wenn sie vielleicht auch wohl etwas klein
sind, eine Einteilung erlauben, die doch wohl als charakteris-
tisch für das Lernen betrachtet werden kann, besonders, da
wir bei jeder unserer Dressuren diese Dreiteilung zurückfinden.

V. ZUSAMMENFASSUNG

Zwei Gruppen Mäuse wurden in einem Labyrinth mit vier
Paaren, sich symmetrisch gegenüberliegenden, in Vorwärts- und
Rückwärtsrichtung (in Bezug auf die allgemeine Laufrichtung)
erstreckenden Sackgassen darauf dressiert, ein Ziel zu suchen,
das in Vorwärts- bezw. Rückwärts-Richtung lag. Jede der beiden
Gruppen wurde erst auf das eine, und dann auf das andere
Ziel dressiert. In der Verteilung der Fehler, die in Tabelle VI
noch einmal zusammengefasst sind, liess sich der Einfluss der
allgemeinen Laufrichtung sowie derjenige der Richtung des
Zieles erkennen, wobei der erste meistens stärker was als der
zweite. Im Verlaufe dieser Dressuren folgten drei Perioden
aufeinander, wie sich in Tabelle V zeigt.

TABELLE VI. Zusammenfassung der Fehlerverteilungen.

	Fehler in den vorwärts gerichteten Sackgassen in %	Fehler in den rückwärts gerichteten Sackgassen in %
Dressur der blauen Gruppe auf das vorwärtige Ziel	61,6	38,3
Dressur der roten Gruppe auf das rückwärtige Ziel	55,2	44,6
Umdressur der blauen Gruppe auf das rückwärtige Ziel	41,7	58,1
Umdressur der roten Gruppe auf das vorwärtige Ziel	67,5	32,4

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NEUE ERGEBNISSE DER ELEKTROPHYSIOLOGISCHEN NERVENFORSCHUNG

VON

B. J. KRIJGSMAN

VORTRAG, GEHALTEN AUF DER JAHRESVERSAMMLUNG DER NED. DIERK. VER.
ZU AMSTERDAM AM 28-1-'39

Die elektrophysiologische Nervenforschung hat die Aufgabe, die Funktion des Nervensystems zu untersuchen. Da diese Funktion sehr viele und verschiedenartige Prozesse umfasst, will ich mich heute beschränken auf die Prozesse, welche bei der Fortpflanzung von Impulsen in den peripheren Nervenbahnen auftreten. Wir stellen somit die Frage: Was geschieht bei der Fortpflanzung eines nervösen Impulses durch einen Nerven?

Eine Lösung dieser Frage erscheint nur möglich, wenn es gelingt, den Impuls und seine Fortpflanzung als messbare Phänomene zu erfassen. Messbare Phänomene können auf verschiedene Weise erzielt werden: Erstens kann man, da die Erregungsvorgänge nicht ohne Energieverbrauch verlaufen können, den Stoffwechsel von ruhenden und aktiven Nerven messen und vergleichen (z.B. Sauerstoffverbrauch, Kohlensäureabgabe, Wärmebildung). Solche Untersuchungen wurden bereits mit grosser Genauigkeit ausgeführt und haben unsere Einsicht in die nervösen Prozesse wesentlich vertieft. Leider ist der Stoffwechsel des Nerven dermassen geringfügig, dass z.B. die Wärmebildung nur deutlich zutage tritt, wenn der Nerv eine gewisse Zeit lang dauernd gearbeitet, d.h. eine grosse Menge von Impulsen geleitet hat. Man misst also auf die Weise die Stoffwechselvorgänge nachträglich als Summe von schon erloschenen Fortpflanzungsprozessen. Wollen wir einen tieferen Einblick in das Wesen der nervösen Erregung erlangen, so müssen wir Messungen vornehmen können während der Bildung und der Fortpflanzung eines einzigen Impulses. Dazu eignet sich nun ein anderes Phänomen, das bei der Aktivität des Nerven auftritt und von elektrischer Art ist: Reizt man einen Nerven, so pflanzt sich über diesen Nerven eine kurze Welle negativer Elektrizität fort. Entstehung und Fortpflanzung dieses

elektrischen Phänomens können wir nun mit Hilfe von physikalischen Apparaten genau messen. Man kann nämlich einen Nerven, also ein Bündel von Axonen (z.B. den N. ischiadicus vom Frosch) aus dem Tier herausnehmen und in eine feuchte Kammer bringen. Darin bleibt der Nerv stundenlang normal wirksam. Die Reizung eines auf diese Weise isolierten Nerven kann verschiedenartig vorgenommen werden: Einmal mechanisch, indem man z.B. einen kleinen Hammer auf den Nerv fallen lässt. Bei solcher mechanischer Reizung werden die Axone jedoch leicht verletzt und überdies ist es kaum möglich die Intensität der mechanischen Reize genügend genau und reproduzierbar abzustufen. Ferner kann man chemisch reizen, indem ein chemisch aktiver Stoff lokal auf den Nerven gebracht wird. Aber auch das ist versuchstechnisch nicht günstig, da solche Stoffe nur langsam eindringen und sich nicht schnell genug wieder entfernen lassen; Reizzeit und Reizintensität beherrschen wir demnach auch bei chemischer Reizung nicht. Es hat sich nun herausgestellt, dass man einen Nerven auf schonende und genau reproduzierbare Weise mit Hilfe eines elektrischen Stromes reizen kann. Legen wir nämlich an zwei Punkten des Nerven die Pole eines Induktionsapparates und lassen wir die Spule arbeiten, so werden dadurch stundenlang normale Impulse im Nerven hervorgerufen. Wollen wir bloss einen einzigen Impuls erzeugen, so applizieren wir einen einzigen Öffnungs- oder Schliessungsschlag, dessen Intensität wir nach Belieben abstufen können. Bei dieser elektrischen Reizung hat man gefunden, dass nicht nur die Höhe des Induktionspotentials, sondern auch die Weise, in der das Potential des Induktionsschlages ansteigt und absinkt, sehr wichtig ist für das Entstehen der nervösen Reaktion. So kann z.B. ein Induktionsschlag mit schnell ansteigendem Potential eine andere Wirkung haben als Schläge mit langsam zunehmendem Potential. Es ist demnach nicht nur die Spannung des Reizstroms, sondern auch seine Form von grosser Wichtigkeit. Mit den üblichen Induktionsapparaten beherrscht man diese Faktore nicht genügend und man benutzt darum neuerdings vielfach kompliziert gebaute Reizapparate mit Gleichrichterröhren, mit Hilfe von denen man den Nerven reizen kann mit Stromstössen von genau abstufbarer Form und Spannung. Dergleiche Reizapparate sind für die Erhaltung von zuverlässigen Resultaten oft unbedingt notwendig.

Der Reiz, den wir dem Nerven geben, trifft ein Bündel von Axonen. Jeder Axon ist bekanntlich von einer Schicht fettartiger Stoff umgeben, der sogenannten Myelinscheide. Der ruhende Axon zeigt nun, wie experimentell nachgewiesen wurde, innerhalb der Myelinscheide (also im Axoplasma) eine negative und an der Aussenseite der Scheide eine positive elektrische Ladung (Abb. 1). Da die innere negative Ladung nicht heraustritt zur Neutralisierung der Aussenseite, so nimmt man an, dass die zwischenliegende Substanz undurchlässig ist für die negativen Teilchen.

Wenn wir nun ein Paar Reizelektroden an die Aussenseite des Axons anlegen, so wird offenbar beim Durchgehen des
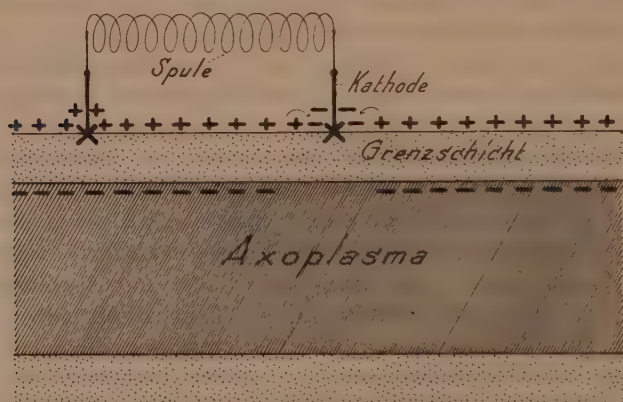
Abb. 1. Schematischer Längsschnitt durch einen Nervenaxon. An der Reizkathode schiesst die negative Ladung aus dem Plasma hervor (Aktionspotential), wodurch benachbarte positive Teilchen angezogen werden (Pfeile).

Reizstroms die Grenzschicht unter der Reizkathode zeitweilig mehr permeabel, weil an dieser Stelle die negativen Teilchen aus dem Axoplasma heraustreten und die positive Ladung der Aussenseite neutralisieren. Dadurch wird die Aussenseite an diesem Punkt elektrisch negativer als in der Nachbarschaft und das verursacht selbstverständlich ein Zuströmen von positiven Teilchen aus den benachbarten Teilen des Nerven (vgl. Abb. 1, Pfeile). Demzufolge werden aber diese benachbarten Punkte der Aussenseite weniger positiv, das elektrische Gleichgewicht wird somit auch da gestört, die Membran wird auch

da permeabel für negative Teilchen, welche heraustreten und die Aussenseite des Axons negativer machen, dadurch strömen wiederum Teilchen aus benachbarten Punkten zu, usw. Das Resultat ist somit, dass alle Punkte der Aussenseite eines Axons nacheinander für kurze Zeit negativer werden, wonach die ursprüngliche Positivität der Ruheladung sich wieder herstellt. Dadurch entsteht eine Welle negativer Elektrizität, die sich über den Axon fortpflanzt (der sogen. Aktionsstrom, Abb. 2). Verschiedene Forscher sind der Meinung, dass dieser Aktionsstrom dem Impuls gleich zu setzen ist und sie betrachten somit den Aktionsstrom als die durch die Nerven übermittelte Erregung. Der Aktionsstrom pflanzt sich mit einer konstanten Geschwindigkeit über das Axon fort (z.B. im N. ischiadicus des Frosches mit 80–100 m pro sec.).

Den Verlauf eines Aktionsstroms können wir genau messen. Wenn wir nämlich ein Galvanometer mit zwei Punkten (P und Q, Abb. 2) des Axons verbinden, so wird beim Fortschreiten des Aktionsstroms in der Richtung der Pfeile zuerst P negativ bezüglich Q und das Galvanometer schlägt somit aus. Beim Weiterschreiten des Aktionsstroms kehrt P wieder zur
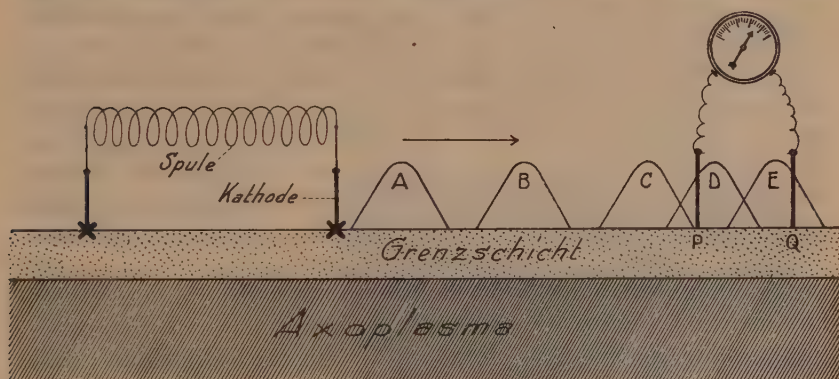
Abb. 2. Fortpflanzung des Aktionsstroms, entstanden an der Reizkathode, über den Axon in die Richtung des Pfeiles. Das negative Aktionspotential ist als Potentialhügel vorgestellt und befindet sich nacheinander in A, B, C, usw.

Ruheladung zurück, aber jetzt erreicht die Welle Q, wodurch Q negativ wird relativ zu P und das Galvanometer schlägt somit nach der anderen Seite aus. Der Aktionsstrom kann demnach als zweiphasischer Galvanometerausschlag registriert

26

werden. Dabei treten aber Schwierigkeiten auf: Wenn die Basis des Aktionspotentialhügels breiter ist als die Strecke P–Q, und das ist sehr oft der Fall, so wird Punkt Q schon negativ bevor P ganz neutralisiert ist. Wir erhalten in solchen Fällen ein falsches Bild des Aktionsstroms, denn das Ende der ersten Phase wird durch den Anfang der zweiten Phase verzerrt. Zur Vermeidung dieser Schwierigkeit wendet man oft ein Verfahren an, wodurch die zweite Phase ganz beseitigt wird: der Axon wird unter dem zweiten Ableitungspunkt abgetötet (z.B. durch Hitze oder Cocaïn). Der Aktionsstrom, der sich nur im lebenden Axon fortpflanzen kann, erlischt nun bevor er den zweiten Ableitungspunkt erreicht und das Galvanometer registriert bloss die negative Schwankung des ersten Ableitungspunktes (sogen. monophasische Ableitung).

Das Potential des Aktionsstroms beträgt nur einige tausendste V. (mV) und dauert nur wenige tausendste Sekunden (msec.). Viele Galvanometertypen sind für solche Messungen ungeeignet; sie sind viel zu träge um den schnellen Verlauf des Aktionsstroms richtig wiedergeben zu können. Ein geeignetes Messinstrument ist das Saitengalvanometer, dessen Prinzip ich kurz erörtern will: Zwischen den beiden Polen eines starken Magneten befindet sich eine Metallsaite von ein bis zwei μ Dicke. Wenn wir durch diese Saite einen elektrischen Strom leiten, so schlägt die Saite im magnetischen Felde nach bestimmten physikalischen Gesetzen aus, und zwar proportional der Stärke des durchgeleiteten Stroms. Mit Hilfe einer starken Lichtquelle und optischer Systeme können wir ein stark vergrössertes Schattenbild der Saite entwerfen und so ihre Schwankungen beobachten. Verbinden wir nun die Saite mit zwei Punkten des Nerven, so wird das Passieren des Aktionsstroms unter diesen Ableitungspunkten einen kurzdauernden Potentialunterschied auslösen, es fliesst ein (gleichfalls kurzdauernder) Strom durch die Saite, diese schlägt somit aus und kehrt wieder zur Ruhelage zurück. Mit Hilfe eines Films, der sich senkrecht zur Richtung der Saitenausschläge bewegt, können wir die Saitenbewegungen photographisch in Kurvenform festlegen.

Das Saitengalvanometer hat unsere Einsicht in das Wesen des nervösen Impulses wesentlich erweitert. Dennoch arbeitet es häufig nicht hinreichend genau, denn die Saite besitzt immer noch eine gewisse träge Masse und folgt schnellen Potentialsprüngen nicht schnell genug zur einwandfreien Registrierung

des Aktionsstroms. Man benutzt daher zurzeit Oszillographen, z.B. Kathodenstrahloszillographen, zur Registrierung der nervösen Impulse. Der Kathodenstrahloszillograph besteht aus einer Vakuumröhre, in der auf die übliche Weise ein Elektronenstrahl erzielt werden kann. Ein solches Strahlenbündel hat nur eine verschwindend kleine Masse und wird also ohne Verzögerung auf elektrische Einflüsse reagieren. Bringt man nun senkrecht zur Richtung des Kathodenstrahles ein elektrisches Feld an, so schlägt der Kathodenstrahl bei Schwankung dieses Feldes aus. Auch der Nervenaktionsstrom, den Polen des elektrischen Feldes zugeleitet, verursacht grundsätzlich solche Schwankungen. Leider sind die Aktionspotentiale zu klein um wahrnehmbare Ausschläge des Kathodenstrahles zu erzielen und man muss daher die Aktionspotentiale durch Verstärkerröhre in geeigneter Weise dermassen ansteigen lassen (manchmal mehr als millionfach), dass sie brauchbare Werte erreichen. Solche komplizierte Verstärkerapparaturen, die in ihrem Bau den Spezialproblemen angepasst werden müssen, stellen dem Forscher schwierige Aufgaben.

Wir wollen jetzt eine Skizze entwerfen von den Resultaten, die wir mit der modernen Reiz- und Registrierungsapparatur erhalten. Erteilen wir dem Axon einen ganz schwachen Reiz, so reagiert er nicht, denn der Registrierungsapparat zeigt keinen Ausschlag und es ist somit kein Impuls entstanden. Lassen wir die Reizstärke allmählich ansteigen, so tritt bei einer bestimmten Reizintensität plötzlich ein Ausschlag auf. Diese Reizstärke hat also einen Impuls ausgelöst, der bei Registrierung mit dem Kathodenstrahloszillographen aussieht wie dies z.B. Abb. 3 zeigt.
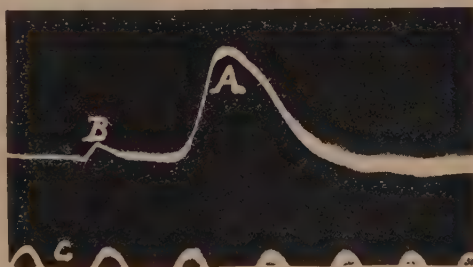
Abb. 3. Aktionsstrom eines Axons aus einem Nerven der Katze. Zeitmarkierung 0,2 msec. (nach GASSER u. GRUNDFEST, 2).

Der ruhende Kathodenstrahl schreibt auf dem horizontal sich bewegenden Film eine wagerechte Linie. Im Augenblick,

in dem der Aktionsstrom den Ableitungspunkt auf dem Axon passiert, schlägt der Kathodenstrahl senkrecht aus und kehrt nachher wieder zur Ruhelage zurück. Dieser senkrechter Ausschlag zeichnet durch die wagerechte Filmbewegung eine Kurve (A). Durch Mitphotographieren einer Wechselspannung mit bekannter Frequenz (Wellenlinie C) erhält man zugleicherzeit eine Zeitmarkierung [1]). Diese Frequenz beträgt in unserem Fall 0,2 msec. und der Aktionsstrom dauert somit 0,4 msec., d.h. die Aktionswelle braucht 0,4 msec. um den Ableitungspunkt auf dem Axon zu passieren.

Das plötzliche Auftreten der Aktionswelle bei einer sehr bestimmten Reizstärke zeigt an, dass der Reiz dann eine gewisse Grenze, eine Schwelle überschreitet, oberhalb welcher der Axon auf den Reiz reagiert. Ist der Reiz kleiner als der Schwellenwert, so geschieht nichts und wenn man den Reiz oberhalb des Schwellenwertes noch weiter ansteigen lässt, so tritt keine Änderung in Grösse und Form des Axonimpulses auf. Ein Axon gibt demnach entweder gar keine Antwort auf den Reiz, oder er reagiert durch Abgabe aller negativer Elektrizität, welche er überhaupt abgeben kann. Das ist das sogenannte Alles-oder-Nichts-Gesetz, welches eine wichtige Rolle spielt bei der Nerventätigkeit. Dieses Alles-oder-Nichts-Gesetz ist selbstverständlich nicht nur gültig im beliebig gewählten Punkt, der elektrisch gereizt wird, sondern in allen Punkten des Axons. Da der an der Reizstelle hervorgerufene Aktionsstrom selbst wieder einen Reiz darstellt für den benachbarten Punkt und dieser Punkt wieder mit ,,Alles'' reagiert usw., so ist ersichtlich, dass der Aktionsstrom sich ohne Verlust durch den Axon, also ,,dekrementlos'', fortpflanzt.

Da der Axon, wenn er auf einen Reiz reagiert, immer alle zur Verfügung stehende Energie abgibt, darf man erwarten, dass er danach einen Augenblick erschöpft sein wird und sich wieder erholen muss zur Restitution des Energieverlustes. Das ist tatsächlich der Fall, denn wenn wir sofort nach einem erfolgreichen Reiz erneut reizen, so reagiert der Nerv nicht auf den zweiten Reiz. Er befindet sich dann in der sogen.

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[1]) Der kleine Hügel B ist, wie man experimentell feststellen kann, ein sogen. Reizeinbruch, verursacht durch Schleifen des Reizstroms; ist somit keine Reaktion des Nerven, sondern ein rein physikalisches Phänomen.

refraktären Periode, während welcher er nicht imstande ist, einen neuen Aktionsstrom zu liefern. Das ist in der Abb. 4 wiedergegeben.

In der Aufnahme 4 dieser Abb. wurden dem Nerven zwei Reize nacheinander gegeben mit sehr kurzem Zeitabstand. Darauf reagiert der Nerv mit einem einzigen Impuls (A); auf den zweiten Reiz kann er nicht antworten, da er sich noch nicht von der ersten Energie-abgabe erholt hat (,,absolut'' refrak-tär). Wird die Zeit zwischen den Reizen etwas grösser (Aufnahme 3), so hat der Nerv sich bereits ein wenig von dem ersten Impuls erholt (,,re-lativ'' refraktär) und gibt schon eine kleine zweite Antwort (A'). Wird das Zeitintervall zwischen den Reizen noch grösser, so ist die Erholung des Nerven weiter fortgeschritten und der zweite Reiz gibt schon eine grössere Antwort (Aufnahme 2). In der Aufnahme 1 schliesslich, bei noch grösserem Reizintervall, ist die Reaktion auf den zweiten Reiz schon etwa gleich gross wie der erste Im-puls. Da die moderne Reizapparatur es ermöglicht, Reize mit genau be-kanntem Intervall zu applizieren, kann die Dauer der refraktären Periode einwandfrei festgestellt wer-

Abb. 4. Refraktäre Periode des N. phrenicus der Katze. Reizintervall bei 1) am gröss-ten, der Nerv antwortet zwei-mal normal. Bei 2), bei klein-erem Reizintervall, ist die zweite Antwort kleiner (rela-tive refraktäre Periode), bei 3) noch kleiner und bei 4) ganz verschwunden (absolute re-fraktäre Periode). Zeitmarkie-rung 1 msec.
(Nach GASSER, 1).

den. Diese beträgt für gewisse Vertebratenaxone etwa eine msec. Das Alles-oder-Nichts-Gesetz und die dazu gehörende refraktäre Periode können bei allen Vertebratennerven mit Myelinscheiden nachgewiesen werden.

Wie ober. gesagt, löst ein unterschwelliger Reiz keinen Impuls aus; es wird nichts geleitet und in einem gewissen Abstand von der Reizstelle beobachten wir keinen Effekt. Lokal jedoch, an der Reizstelle selbst, geschieht doch etwas. Ein unterschwelliger Reiz hinterlässt nämlich an der Reizstelle

eine gewisse negative Ladung [1]). Von diesen lokalen Prozessen gibt Abb. 5 eine Skizze:

Ein schwacher unterschwelliger Reiz ladet den Axon einfach physikalisch lokal an der Reizstelle (R) ein wenig negativ auf; es entsteht somit ein kleiner „Potentialhügel" (a_1), der sich nicht fortpflanzt. Stärkere unterschwellige Reize hinterlassen grössere negative Ladungen (a_2, a_3); die aufgebrachte Ladung ist der Reizgrösse proportional. Ist diese Ladung gross genug, so entsteht ein Impuls, d.h. also, infolge der aufgebrachten Ladung wird die Membran des Axons durchlässig und die innere negative Ladung kommt ganz heraus. Dadurch steigt plötzlich die Negativität an der Reizstelle noch viel mehr; das ist der Aktionsstrom, der sich fortzupflanzen beginnt, da dieser imstande ist auch benachbarte Nerventeile genügend stark zu aktivieren (Abb. 5, $I \rightarrow I_1$). Wir können also die Schwelle durch Linie
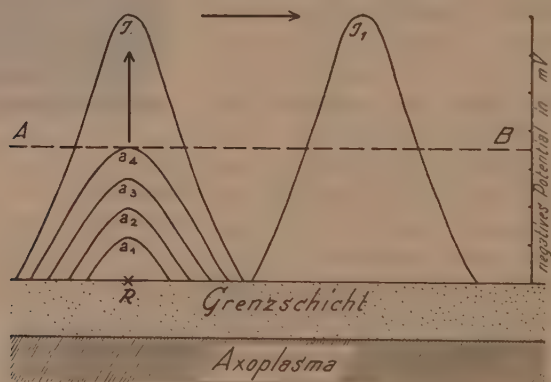
Abb. 5. Die Entstehung des Lokalpotentials an der Reizstelle R bei verschiedenen Reizstärken. Bei zunehmender Reizstärke ist die vom Reiz hinterlassene negative Ladung entsprechend grösser (a_1, a_2, a_3) und erreicht bei einem noch stärkeren Reiz die Schwelle AB. Dann schiesst die negative Plasmaladung heraus (Impuls I), die sich fortzupflanzen beginnt (I → I_1 usw.).

AB wiedergeben. Ist das Potential der durch den Reiz aufgebrachten Ladung gross genug (a_4), so wird die Schwelle erreicht und der Impuls schiesst aus dem Axon heraus. Ist die Aufladung kleiner (a_3—a_1), so löst sie keinen Impuls aus und

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[1]) Diese Ladung darf nicht verwechselt werden mit dem gleichfalls negativen Aktionsstrom.

die aufgebrachte Ladung geht in wenigen msec. wieder ver-
loren, indem sie abfliesst (neutralisiert wird durch die benach-
barten positiven Stellen).

Durch die Messung von Art und Zeit, in der die durch den
unterschwelligen Reiz aufgebrachte negative Ladung wieder
abfliesst, sind interessante Vorgänge zutage getreten. Diese
unterschwelligen Pro-
zesse wollen wir jetzt
näher betrachten. Im
Schema der Abb. 6
ist horizontal die Zeit
in msec., vertikal das
Potential der durch
den unterschwelligen
Reiz an der Reizstelle
hinterlassenen negati-
ven Ladung angege-
ben. Misst man nun
den Potentialverlauf
an der Reizstelle selbst,
so stellt sich heraus
(Kurve a): Bei ganz
schwacher Reizung
steigt das Potential
während des Reizes
(Reizdauer etwa 0,1
msec., schwarzer
Block) schnell an,
nimmt nach dem Ende
des Reizes in Form ei-
ner Exponentialkurve
ab und ist nach etwa
2 msec. wieder ganz verschwunden[1]. (Untersuchungen von HILL,
3 und KATZ 6). Bei stärkeren Reizen tritt zunächst ein vollkommen
ähnlicher Potentialverlauf auf (Kurve b und c). Bei noch weiter
zunehmender Reizstärke jedoch (immer noch unterschwellig!)
sehen wir, wie die aufgebrachte Ladung nicht mehr in Form
einer Exponentialkurve abfliesst, sondern es entsteht eine Aus-

Abb. 6. Zu- und Abnahme der negativen
Ladung eines Axons an der Reizstelle. Hori-
zontal: Zeit in msec., vertikal: Ladungspoten-
tial. Reizdauer etwa 0,1 msec. (schwarzer
Block). Während des Reizes steigt die Ladung
schnell und stark an und fällt nachher in Form
einer Exponentialkurve ab (a, b und c). Bei
stärkeren Reizen wird die Abweichung vom
exponentiellen Abfall immer deutlicher
(d, e und f).

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[1] D.h. in 2 msec. hat sich wieder das ursprüngliche positive Ruhepoten
tial der Axonaussenseite hergestellt.

buchtung in der Kurve (d und e, Katz 6, Ledingham and Scott 9, Hodgkin 4) oder sogar ein vorübergehender Anstieg (Kurve f). Einen solchen temporären Anstieg habe ich (Krijgsman 7) gefunden bei einem Nerven von *Helix pomatia*, was aus der Abb. 7 hervorgeht. In dieser Abbildung ist horizontal die Zeit in msec. und vertikal die durch den unterschwelligen Reiz auf den Nerven gebrachte Ladung eingetragen. Es ist ersichtlich, dass während des Reizes eine gewisse negative Ladung auf dem Nerven entsteht, der nach Ablauf des Reizes nicht in Form von einer Exponentialkurve (A) abnimmt, auch nicht nach der ausgebuchteten Kurve B, sondern zuerst noch eine Zeit lang weiter anwächst, um erst später abzusinken.

Abb. 7. Lokalpotential beim N. intestinalis von Helix pomatia. Das Potential steigt bis zu 8 msec nach dem Reiz stark an und sinkt erst dann allmählich ab.

Dieses Anwachsen der aufgebrachten Ladung nach Beendigung des Reizes ist ein sehr interessanter Befund. Ein Kondensator, den man aufladet, wird sich ja nach Unterbrechung des Ladungsstroms nicht noch weiter aufladen! Wir können deshalb mit Recht vermuten, dass ein starker unterschwelliger Reiz im Axon mehr herbeiführt als eine rein physikalische Aufladung. Tatsächlich hat sich herausgestellt, dass die Ladungskurve nach starken unterschwelligen Reizen aus zwei Komponenten besteht (Hodgkin). Das ist in der Abb. 8 schematisch wiedergegeben. Die Kurve a ist der gemessene Potentialverlauf an der Reizstelle nach einem starken unterschwelligen Reiz; sie zeigt die schon erörterte Ausbuchtung. Diese Kurve ist, wie experimentell festgestellt wurde, die Resultante von zwei anderen Kurven, und zwar von der rein physikalischen Ladungskurve (b), die wir bei schwächeren Reizen kennen lernten. Dieses physikalische „Lokalpotential” erweckt nun, wenn es stark

genug ist (aber immer noch unterschwellig), einen physiologischen Prozess, der ebenfalls lokal beschränkt bleibt. Dieser physiologische Prozess produziert ebenfalls negative Elektrizität (lokale Antwort, Kurve c). Es ist ersichtlich, dass dieser Prozess eine gewisse Zeit zu seiner Entwicklung braucht, da er erst einige msec. nach Ablauf des Reizes seinen Höhepunkt erreicht. Die Summe des physikalischen und physiologischen Potentials ergibt die gemessene Kurve a.

Wir können den Verlauf der unterschwelligen Prozesse folgendermassen zusammenfassen: Ein schwacher Reiz deponiert bloss eine kleine physikalische Ladung auf die Reizstelle, welche wieder verloren geht. Je stärker der Reiz ist, desto grösser ist diese Aufladung. Schliesslich ist die Aufladung so gross, dass sie den Nerven lokal zu aktivieren anfängt. Die Grenzschicht des Axons beginnt dann nämlich schon etwas durchlässig zu werden und es treten bereits einige negative Teilchen aus dem Axlopasma heraus. Bei weiterer Verstärkung des Reizes wird auch diese lokale Antwort grösser, bis schliesslich physiologische und physikalische Ladung zusammen genügend gross sind um die Reizschwelle zu erreichen, d.h. die Permeabilität der Grenzschicht steigt plötzlich weiter an und lässt die gesamte negative Elektrizität aus dem Innern entweichen. Das ist der Aktionsstrom, der sich darauf nach dem Alles-oder-Nichts-Gesetz dem Axon entlang fortpflanzt. Der Nerv wird sozusagen

Abb. 8. Die Kurve a stellt den festgestellten Verlauf der negativen Ladung an der Reizstelle nach einem starken unterschwelligen Reiz dar. Diese Kurve entspricht der Summe der physikalischen Ladung (Lokalpotential, Kurve b) und der lokalen Antwort des Axons (Kurve c). Horizontal Zeit in msec, vertikal Potential. Schwarzer Block = Reizdauer.

durch den Reiz lokal erhitzt (Lokalpotential), fängt bei genügend starker Reizung an zu glimmen (lokale Antwort), aber erst wenn die Erhitzung stark genug ist, entflammt der Axon wirklich (Impuls) und die Flamme läuft über den Axon weiter.

Beim N. ischiadicus des Frosches hat die maximale lokale Antwort (d.h. diejenige lokale Antwort, welche zusammen mit dem Lokalpotential die Schwelle erreicht), ein Potential von ein bis zwei mV und ist einige mm breit, stellt also einen kleinen Potentialhügel dar. Es ist nun dem englischen Forscher
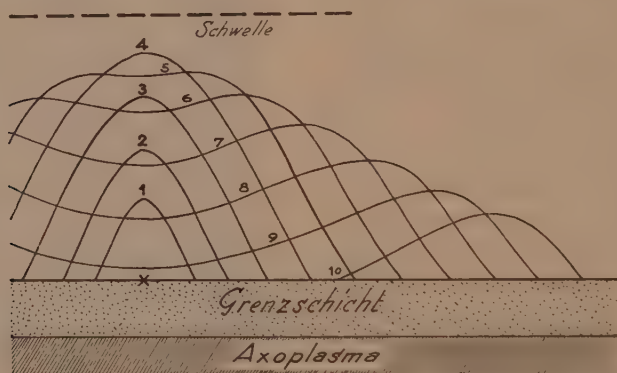
Abb. 9. Verlauf der lokalen Antwort bei künstlich erhöhter Schwelle. Die lokale Antwort entsteht an der Reizstelle (x) und wächst in der Reihenfolge 1, 2, 3, 4 an. Darauf nimmt sie in der Mitte wieder ab, während peripher die Ausbreitung weitergeht (5, 6, 7, 8, 9 und 10).

Hodgkin gelungen, bei Beinnerven von Crustaceen die Schwelle durch Behandlung mit Salzen zu erhöhen, das heisst die Fähigkeit des Nerven zur Lieferung von Impulsen zu verringern, jedoch ohne Störung der lokalen Antwort. Unter solchen Umständen hat er beobachtet, dass die lokale Antwort bei steigender Reizstärke grösser und grösser wird; ein Impuls entsteht aber nicht, da das Gesamtpotential die erhöhte Schwelle nicht erreichen kann. Schliesslich ist die lokale Antwort einige cm breit und ihr Gipfel gleich hoch wie sonst der Impuls; sie ist jedoch kein Impuls, da sie sich nicht fortpflanzt. Hodgkin hat sogar festgestellt, dass der Potentialhügel an der Reizstelle bereits wieder absinken kann, während er sich links und rechts noch ausbreitet, wie aus der Abb. 9 ersichtlich. In dieser Abb.

ist Entstehung und Abnahme der lokalen Antwort bei künstlich erhöhter Schwelle wiedergegeben. An der Reizstelle entsteht der Potentialhügel (1), wächst über 2 und 3 an zu 4 und dann fängt der Gipfel bereits an abzusinken (5), während das Potential sich links und rechts noch ausbreitet. Das ist in den nächsten Stadien 6, 7, 8 und 9 noch stärker ausgeprägt und schliesslich (10) ist nur noch ein kleines Potential vorhanden in einigem Abstand von der Reizstelle. Die lokale Antwort ist in diesem Fall eine S c h e i n w e l l e; in verschiedenen cm Abstand von der Reizstelle ist sie noch messbar; jedoch kleiner und kleiner nach Massgabe ihrer Entfernung von der Reizstelle.

Zusammenfassend können wir schliessen, dass im Axon zur Fortpflanzung der Erregung zwei biologische Prozess notwendig sind: Erstens die lokale Antwort, welche unter normalen Umständen klein bleibt und vollkommen abhängig ist von der Reizstärke, also nicht dem Alles-oder-Nichts-Gesetz gehorcht. Durch diese lokale Antwort wird ein zweites Phänomen ausgelöst, nämlich der Impuls, der immer gleich gross und unabhängig von der Reizstärke ist (Alles oder Nichts) und sich ohne Verlust durch den Axon fortpflanzt.

Vorstehende Resultate wurden erhalten an Vertebraten- und Crustaceennerven. Bei niederen Tieren dagegen ist das Alles-oder-Nichts-Gesetz bei der Erregungsleitung offenbar nicht immer gültig. Nehmen wir z.B. ein Protozoen, zumal eine Amöbe mit einem langen Pseudopodium, und reizen wir dieses peripher, so ist Stärke und Ausbreitung der Kontraktion des Pseudopodiums erheblicher, je nachdem der Reiz stärker war. Da Protozoen kein Nervensystem besitzen, welches die Reaktionsgrösse regulieren kann, so müssen wir wohl annehmen, dass der Impuls [1]) selbst schwächer wird während seiner Fortpflanzung und weiter von der Reizstelle entfernt eine immer schwächere Kontraktion auslöst. Wenn nämlich die Reizübertragung hier ohne Verlust stattfände, so müsste auf jeden schwachen Reiz hier immer das ganze Tier reagieren.

Weiter ist die Ungültigkeit das Alles-oder Nichts-Gesetzes nachgewie.en worden in Gastropodennerven, bei Aplysia mit

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[1]) Ich spreche kurzheitshalber auch bei Protozoen von ,,Impulsen", obgleich ihre direkte Messung da noch aussteht. Eine Reizübertragung muss aber auch bei Protozoen stattfinden.

Narkoseversuchen durch JORDAN und LULLIES (5) und bei Helix pomatia elektrophysiologisch von mir (KRIJGSMAN 7, 8). In den Nerven von Helix wird der Impuls nämlich während der Fortpflanzung ständig kleiner. Das ist ersichtlich, wenn wir den Impuls in verschiedenen Abständen der Reizstelle registrieren, d.h. ihn messen, wenn er verschieden lange Strecken zurückgelegt hat. Solche Messungen zeigen die Aufnahmen der Abb. 10. In diesen Aufnahmen tritt aber noch ein anderes Phänomen zutage, das ich zuerst erörtern will: Bei diesen Versuchen wurde der ganze Nerv (N. intestinalis) gereizt, also nicht ein einziger Axon. Der Aktionsstrom ist also zusammengesetzt aus der Summe der Aktionsströme der Axone. Die Axone leiten aber ihre Aktionswellen nicht alle gleich schnell und die Fortpflanzung ähnelt demnach einem Wettlauf: Die Läufer fangen zwar alle zur gleichen Zeit und im selben Punkt an, wenn sie jedoch eine gewisse Strecke zurückgelegt haben, so haben die Schnellsten schon einen kleinen Vorsprung und die weniger Schnellen bleiben, oft gruppenweise, etwas zurück. Das ist in der Abb. 10 sehr deutlich zu sehen. Die Aufnahme

Abb. 10. Der Aktionsstrom im N. intestinalis von Helix pomatia, aufgenommen in verschiedenen Abständen von der Reizstelle: 1 = einige mm, 2 = 1 cm, 3 = 2 cm von der Reizstelle. a = Reizeinbruch.

1, auf einige mm Abstand von der Reizstelle, zeigt einen zweigipfeligen Aktionsstrom: Voran geht eine steile Welle, zusammengesetzt aus den Aktionsströmen einer Anzahl von schnell leitenden Fasern. Aber bereits beim Durchlaufen dieser kleinen Strecke sind die Aktionswellen der langsameren Axone etwas zurückgeblieben und bilden zusammen eine zweite Welle. Misst man den Impuls in einem Abstand von 1 cm von der

Reizstelle (Aufnahme 2), so sind die Wellen weiter auseinander-gerückt und breiter geworden. Wenn wir schliesslich den Impuls registrieren nach Zurücklegung einer Strecke von etwa 2 cm (Aufnahme 3), so sind die zwei Gipfel breit ausgezogene Hügel geworden und sie fangen an, sich in kleinere Gipfel zu zerteilen. Also genau wie Wettläufer, welche im Laufe des Wettrennens immer mehr auseinander rücken und allmählich kleine Gruppen bilden. Durch diese Unterschiede der Axonen-geschwindigkeit kann man bei Aufnahmen des Aktionsstroms eines ganzen Nerven nicht die Potentialhöhe als Masstab für die Gültigkeit des Alles-oder-Nichts-Gesetzes verwenden. Ist dieses Gesetz gültig, so muss aber die Gesamtoberfläche des Aktionsstroms in allen Abständen von der Reizstelle gleich bleiben, da die Oberfläche ein direktes Mass ist für die geleitete Elektrizitätsmenge. In Vertebratennerven bleibt diese Ober-fläche tatsächlich konstant. Messen wir aber die Oberflächen der Impulse im Helixnerven, so stellt sich heraus, dass sie sich in Abb. 10 verhalten wie 100 in der Nähe der Reizstelle (Auf-nahme 1) zu 92 eine Strecke weiter (Aufnahme 2) und etwa 80 in der Aufnahme 3. Im untersuchten Nerven von Helix bleibt die geleitete Energiemenge während der Fortpflanzung somit nicht konstant; der Impuls nimmt unterwegs fortwährend an Intensität ab. Das Alles-oder-Nichts-Gesetz ist hier nicht gültig, die Leitung zeigt also einen Verlust (Dekrement). Ist der Reiz genügend stark, so wird der Axon an der Reizstelle alle negative Elektrizität abgeben, die er abgeben kann; das gilt aber nur an der Reizstelle selbst, denn unterwegs tritt Verlust auf, d.h. an weiteren Stellen des Axons wird nicht alles abgegeben. Ist der Reiz kleiner, so gibt der Axon an der Reizstelle nicht alles und die Erregung wird unterwegs früher erlöschen müssen. Tatsächlich habe ich feststellen können, dass bei schwachen Reizen der Impuls nur in der Nähe der Reiz-stelle messbar und weiter entfernt nicht mehr wahrnehmbar ist. Je stärker der Reiz, desto weiter kommt die Erregung; maximale Reize lösen eine maximale Höhe (d.h. maximales Potential) und maximale Ausbreitung der Erregung aus.

Entwirft man von diesen experimentellen Befunden ein schematisches Bild, so stimmt dieses vollkommen überein mit der Abb. 9, in der die Entwicklung der lokalen Antwort im Crustaceennerven bei künstlich erhöhter Schwelle wiederge-geben wurde. Das bedeutet, dass die sogenannte Fortpflanzung

mit Dekrement bei den Gastropoden in allen bekannten Eigen-
schaften vollkommen übereinstimmt mit der lokalen Antwort
des Alles-oder-Nichts-Nerven. Im Helixnerven pflanzt sich ja
die Erregung weiter fort und das Potential ist höher, je nachdem
der Reiz stärker ist. Das ist bei der lokalen Antwort genau so.
Beide sind in Höhe und Länge vollkommen abhängig von der
Reizstärke: Bei beiden wird die Ausbreitung vollständig be-
stimmt durch das Potential, welches an der Reizstelle ausgelöst
wird; beide können an der Reizstelle schon wieder verschwinden
bevor die endgültige Ausbreitung erreicht ist und sind demnach
Scheinwellen (Scheinimpulse). Ein Axon von Helix arbeitet
m.A. wie ein Crustaceen- oder Vertebratenaxon, dessen Schwelle
unerreichbar hoch liegt und in dem also keine echte Impulse
ausgelöst werden können. Das, was wir bis jetzt bei niederen
Tieren als eine Fortpflanzung mit Dekrement aufgefasst haben,
ist also keine misslungene oder unvollkommene Alles-oder-
Nichts-Fortpflanzung, sondern eine grosse lokale Antwort, die
sich weit nach links und rechts ausbreitet und zuletzt Muskel-
kontraktion auslöst. Bei niederen Tieren vermittelt der Nerv
offenbar ihre Signale mit Hilfe von solchen beschränkten
Reaktionen; bei Crustaceen und Vertebraten hat sich hierzu
noch ein zweites Phänomen gestellt, die Alles-oder-Nichts-
Fortpflanzung.

Zwischen Bau und Funktion des Axons kann man, vorläufig
nur skizzenhaft, einen interessanten Zusammenhang aufdecken,

Tiergruppe	Myelin-scheide	Beschränkte Reaktion	Alles-oder-nichts	Zentren-entwick-lung
Protozoen	—	+ +	—	—
Gastropoden	±	+ +	—	±
Crustaceen	+	+	+	+
Vertebraten	+ +	±	+ +	+ +

was in der Tabelle wiedergegeben ist. Betrachten wir zuerst
die Entwicklung der Myelinscheide, so sind wir klar darüber,
dass ein Pseudopodium einer Amoebe sicherlich nicht umgeben
wird durch eine markscheidenähnliche Hülle. Bei Gastropoden

wissen wir vom axonalen Bau noch sehr wenig; ich habe aber feststellen können, dass daselbst keine ausgeprägte Myelin-scheide vorkommt; der Axon ist noch nicht viel mehr als ein undifferenzierter Plasmafaden. Bei Crustaceen liegt schon eine Differenzierung im axonalen Bau vor, denn bei diesen Tieren sind nach den Untersuchungen von SCHMITT und BEAR (11) sowie von YOUNG (12) die Axone umgeben von einer dünnen Schicht gerichteter Lipoid- und Proteinmoleküle, welche als eine Markscheide in erster Anlage aufgefasst werden kann. Bei den motorischen Vertebratennerven schliesslich ist die Markscheide bekanntlich gut entwickelt.

Die „Fortpflanzung mit Dekrement", also die beschränkte Reaktion, ist bei Protozoen die einzige Weise, auf die eine Erregung sich ausbreiten kann. Auch bei Gastropoden wurde biss jetzt ausschliesslich eine beschränkte Reaktionsausbreitung festgestellt. Bei Crustaceen ist die beschränkte Reaktion noch ziemlich gross, daneben finden wir da jedoch schon einen echten Impuls mit Alles-oder-Nichts-Fortpflanzung, obgleich nach manchen Autoren (z.B. LULLIES 10) in langsamen Nervenfasern von Crustaceen doch vielleicht eine Leitung mit Dekrement vorkommt. Bei Vertebraten ist die beschränkte Reaktion zu einem kleinen Rest reduziert und die Alles-oder-Nichts-Leitung ist daselbst zur vollständigen Entwicklung gekommen.

Es ist demnach nicht zweifelhaft, dass die Entwicklung des Axonbaus zusammenhängt mit dem Auftreten der Alles-oder-Nichts-Leitung und mit der Abnahme der beschränkten Reak-tion. Mit diesem Entwicklungsbild ist andererseits die Rolle der nervösen Zentren innig verbunden:

Bei Protozoen gibt es keine nervöse Zentren; zentrale Regu-lierung und Hemmung von Reizeinflüssen ist da nicht möglich und alles muss daher bei diesen Tieren in der Peripherie regu-liert werden. Bei Gastropoden sind gleichfalls die Zentren noch nicht genügend entwickelt zur vollständigen Regulierung der Reflexe. Es ist demnach auch hier notwendig, dass im Nerven-axon selbst die Erregung während der Übertragung allmählich geringer wird, denn bei einer Alles-oder-Nichts-Leitung würde das Tier viel zu stark und allgemein auf schwache lokale Reize reagieren. Bei den höheren Tieren schliesslich sind die Zentren soweit entwickelt, dass sie imstande sind die Reaktionsstärke genau abzustufen und zu regulieren. Dadurch wird aber eine Erregungsabstufung in den peripheren Nerven überflüssig und

kann eine Alles-oder-Nichtsleitung auftreten, wobei der Axon degradiert wird zu einem Telegraphendraht, welcher den Impuls genau so weiter führt, wie er ihn empfangen hat.

Die Entwicklung der Funktion der Nervensystems zeigt demnach, im Zusammenhang mit dem Bau, einige grosse Linien: Degradierung der regulierenden Funktion im peripheren Nerven und Verschiebung dieser Regulierung nach den sich immer mehr vervollkommnenden Zentren. Diese Evolution wird ermöglicht durch die Differenzierung des axonalen Baues, welcher sich von einem einfachen Plasmafaden entwickelt zu einem Axon mit Markscheide, der den Impuls ohne Verlust leitet.

Aus dem Vorstehenden kann man schliessen, dass auch die vergleichende Physiologie imstande ist, Evolutionslinien im Tierreich aufzudecken. Nicht nur die Form, sondern auch die Funktion entwickelt sich bei den verschiedenen Tiergruppen zu immer höheren Stufen, und gleichwie nicht mehr benutzte Organe degenerieren und zu Rudimenten verkümmern, so gibt es auch Rudimente von verschwindenden Funktionen. Im vorliegenden Fall ist die lokale Antwort des Vertebratenaxons ein solcher Rest der normalen Fortpflanzungsweise in den Nerven von niederen Tieren. Während die lokale Antwort bei niederen Tieren die Aufgabe hat, an und für sich die Erregung zu übermitteln, ist sie bei höheren Tieren reduziert zu einem lokalen Prozess, der keine Signale mehr übermitteln kann, sondern nur noch nützlich ist als Schrittmacher für eine neue, höhere Form der Signalübertragung, nämlich für den Impuls mit der Alles-oder-Nichts-Leitung.

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THE BRACONID ALYSIA MANDUCATOR PANZER IN ITS RELATION TO THE BLOW-FLY CALLIPHORA ERYTHROCEPHALA MEIGEN

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CONTENTS

CHAPTER I

INTRODUCTION

Alysia manducator Panzer belongs to the family Braconidae, of the order Hymenoptera. The imagines of this species are free-living and winged. Head, thorax and abdomen are black, but the legs are red. The length from the front of the head to the end of the abdomen varies from fully 4 mm to 7 mm (cf. fig. 13). The sexes can be distinguished by the ♂♂ being much more lively in their movements and having a more slender abdomen and longer antennae. The number of joints of the flagellum of the antennae in the ♂♂ is usually 42, but may be anything between that and 36, while in the ♀♀ it is commonly 31, varying between 29 and 33. It is not always the same in both the right and left antenna. In the ♀ the point of the ovipositor is always visible on the ventral side of the abdomen.

The larva lives as internal parasite in the larvae of other Insects found in putrifying meat, principally in the carnivorous maggots of Diptera.

The behaviour of parasite and host has been described by GRAHAM SMITH (1916 and 1919), ALTSON (1920), MYERS (1927 and 1929) and others. For the sake of completeness I will give a brief repetition of it here and in doing so some of the respects in which I differ from the above mentioned authors can be discussed, chiefly concerning the host.

1. Behaviour of the parasite.

Alysia manducator seems to find its prey guided by the smell coming from putrifying meat, which leads it to an environment where maggots are likely to be found (LAING, 1937). Thus it may be present at the source of food before the fly eggs laid there have come out (MYERS, 1927). It explores the food with groping movements of the ovipositor, which does not yet leave the ovipositor-sheath. When a moving maggot is found it is stung, whereby the ovipositor moves rapidly in and out in the direction of its length and then becomes still, while during this process the ovipositor-sheath is folded back parallel to the abdomen. At each sting one egg at most is laid, but large numbers of maggots are attacked one after another, in which the *Alysia* ♀ displays a dogged persistance.

The eggs hatch in the maggots and the larva develops further

within the host. During the process the host proceeds to pupation. The parasite then increases rapidly in size and finally devours the soft parts of the host entirely. It lies for some time in the empty puparium of the host and then spins a cocoon inside it within which it pupates. During the last larval stage, as pupa and as imago the parasite always lies with its head towards the anterior end of the host puparium. An occasional exception to this has been observed (ALTSON), but never occurred during my research. The imago seems to remain in the puparium for a few days before emerging and has then, like the pupa, a greatly distended abdomen, caused by the size of the intestinal tube. As the mid-intestine during the larval stages is closed caudally so that the contents of the intestine cannot be ejected, the diameter is greatly increased. The contents of the intestine has gradually become hardened into a solid rod-like object, the meconium, which is only ejected when the imago hatches. The emergence of *Alysia* from the strong puparium of the host occurs by means of the powerful mandibles, which move in an outward direction, a characteristic of the Alysiidae, and break out an opening in the wall of the puparium. Sometimes the cap of the puparium bursts open during the process, along the preformed ring-like rupture at the anterior end, and remains hanging to the lining produced by the parasite. In most cases, however, the opening made by *Alysia* is of irregular shape. The parasite struggles through the narrow aperture, causing so much pressure on the abdomen that the meconium is released and usually remains behind in the puparium of the host. After this the parasite is immediately ready to fly, the wings are fully expanded at the moment that it emerges from the puparium.

The two sexes are almost immediately ready to copulate. The ♀♀, as soon as they come into the neighbourhood of maggots, even before they are fertilized, begin readily to oviposit, while depositing eggs, which then develop partheno-genetically (GRAHAM SMITH, ALTSON).

2. Behaviour of the host.

When the ♀ of *Alysia* pierces the cuticle of a maggot with its ovipositor, the first reaction of the host is vomiting and voiding. These symptoms, however, are often hardly perceptible, as the ejected substances are frequently almost colourless and quickly spread over the substratum or, as regards the faeces, the posterior

end of the maggot. It then begins to struggle violently, but when the ovipositor is once inserted the parasite is almost always able to hold its prey fast, although it may be violently swung hither and thither. A paralysis of the victim then follows, which is according to ALTSON (1920) caused by a poison secreted by accessory glands and injected into the host during oviposition. MYERS (1927) stated that during the paralysis the contractions of the dorsal vessel of the maggot cease and only after some time regain their original frequency. The duration of the paralysis seems to depend in the first place upon the kind of host. In maggots of *Calliphora erythrocephala* Meig. it lasts 1 to 2 minutes. In laboratory experiments I observed that the size of the maggot might effect the length of the paralysis; in smaller maggots it lasted for a shorter time than in larger ones of the same species. When an *Alysia* ♀ stings many times in succession the action of the poison seems to become less violent and the paralysis is then shorter or less complete. The paralysis in many cases is followed by convulsive movements of the maggot in which strong waves of contraction run through it.

Species that may be used as host are given in literature as:

Diptera: *Lucilia caesar* L., *L. sericata* Meig., *Calliphora erythro-cephala* Meig., *C. vomitoria* L., *Muscina stabulans* Fall., *Hydrotaea dentipes* F., *Phormia groenlandica* Ztt. and the Coleopteron: *Creophilus maxillosus* L.

MYERS (1927, p. 220) comments upon this as follows: "Tax-onomically the host-preferences of *Alysia manducator* are some-what diverse; ethologically they are remarkably restricted". On the basis of parasitization percentages of different sets he comes to the conclusion that the various kinds of host are not accepted indescriminately, but that *Alysia* certainly has its preference. Maggots of *Lucilia* would be preferred to those of *Calliphora*.

It may occur, however, that a parasite is especially attracted to the species in which it has itself gone through its larval development (cf. SALT, 1935). In this case, then, the tendency to choose a particular host may not be due to the species as such, but is a peculiarity of the individual. As the parasitization may take place even through a layer of food and as the parasite seldom releases a maggot when once attacked it appears to me that the host-selection by *Alysia* is not decisive but that the behaviour of the maggots may give indications of whether they

are to be parasitized or not. If, for instance, one kind of maggot or one stage of developmment is more photophobic than another, the parasitization percentage of the latter may be higher without this being the consequence of a definite choice on the part of the parasite. I did not pursue this question further, however, as for simplicity I worked entirely upon one host, *Calliphora erythrocephala* Meig.

Concerning the size of the host at parasitization it has been stated that *Alysia* prefers maggots that are "fully fed" and "fullgrown", while it despises small maggots. My experiments are to a great extent in conflict with these statements. Small maggots on the bait were certainly attacked, especially of course when no larger ones were present. In the laboratory it occurred frequently and even when larger and smaller maggots were offered at the same time [1]). It seems to me that *Alysia* descriminates very little between maggots of varying size. It is possible that when they crawl together on a cadaver the larger specimens feed more on the surface and would therefore be more parasitized. I consider it, like the parasitization of different species of host, to be due more to the behaviour of the maggots than to a special preference of *Alysia*.

A third matter which the behaviour of the host may effect, is the place where the ovipositor is inserted. In searching for parasite eggs in maggots I found them principally in the posterior segments. On the other hand I frequently observed cases in which the attack of the parasite was directed towards the head-regions of the maggot. I assume that the anterior, thinner part of the maggot, as it is more mobile, is more likely to evade the ovipositor. In many cases an attempt to sting in the anterior segments will result in an insertion of the ovipositor in the posterior parts.

CHAPTER II

CATCHING AND CULTIVATING PARASITE AND HOST

1. Catching and cultivating of imagines.

In the vicinity of the Zoological Laboratory in Leiden, the bodies of lesser mammals, such as dogs and cats were laid in

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[1]) Once a maggot of 4 mm that had crept amongst larger individuals was attacked and remained attached to the ovipositor of the ♀ when it was withdrawn. The ♀ moved about for sometime "very awkwardly" with it and finally removed it with its hindmost pair of legs.

a packing case, about 1 by 2 m in size, in which there was a layer of soil of about 25 cm deep. The bodies were cut open ventral medial and the viscerals removed as being subject to too rapid putrifaction. In a few days the cadavers began to decay. The cases were covered by sacks laid upon wire netting and it was observed that the influence of the atmosphere being partially excluded, putrefaction was less rapid. Numerous insects were attracted by this bait. The most conspicuous were:

Diptera: *Calliphora erythrocephala* Meig., *Lucilia sericata* Meig., *L. caesar* L., *Sarcophaga* sp., *Musca domestica* L.

Hymenoptera: *Alysia manducator* Pz., *Vespa* sp., (*Atractodes* sp.).

Coleoptera: *Necrophorus humator* F., *Silpha* sp.

Chiefly *Calliphora erythrocephala* deposited eggs on the rotting substance. The maggots remained in the case if care was taken that there was food enough, so that the pupae could be collected later out of the earth.

For hygienic reasons I tried replacing the animals by waste matter from the Municipal Abattoir, but this consisted largely of sinews and other material which the maggots did not eat. The consequence was that too large a number of eggs was laid in proportion to the nourishment in the substance ("over-blowing", cf. SALT, 1930; HOLDAWAY, 1930; SALT, 1932). This might give rise to the parasites chancing upon insufficiently nourished or unhealthy maggots, which I considered undesirable. Moreover, as the maggots roved in search of food, a great many escaped from the case, which would lead to the loss of a great deal of parasitized material. I therefore decided to adhere exclusively to my first bait.

Covering the case with wire netting and sacks, besides reducing the rate of putrefaction, had the further good effect, that the "shady situation" mentioned by GRAHAM SMITH (1919, p. 376) was approached, under which conditions a higher percentage of parasitization of *Calliphora erythrocephala* by *Alysia manducator* might be expected.

Both the ♀♀ of *Alysia* and the ♂♂, which are more lively and quick, could be easily caught in a sucking-tube. I used a good sized instrument in which the sudden stream of air was caused by an india-rubber ball. It proved to be unnecessary to construct special traps (cf. GRAHAM SMITH, 1916).

From the middle of May to certainly the end of September ♀♀ of *Alysia* were found on the bait, where they were usually

occupied in laying eggs. It was only very seldom that I observed a ♀ anywhere else except on the bait, which, on the other hand, was hardly ever visited by the ♂♂. The ♂♂ were found somewhat earlier (in 1939, from April 27th), and in fine weather were usually found on the plants (*Petasites officinalis* Mnch., *Anthriscus silvestris* Hoffm.) round about the packing-case, where they collected in great quantities but never at more than 3 m distance from the bait.

Parasites could be obtained not only by catching the imagines of *Alysia* but by collecting the pupae of *Calliphora* in the case at regular intervals, amongst which parasitized specimens were also found. These parasites, emerging in the laboratory were in some respects of more value for cultivation than those caught in the open air, as, in contrast to the latter, certain data such as age, fertilization of the ♀ and number of egg-depositions could be accurately ascertained.

The imagines of *Alysia* hatched in the laboratory and those caught in the open air were put separately into an experimental cage of 25 × 25 × 33 cm, with walls of wire gauze. In these cages, where ♂♂ and ♀♀ were put together, glass dishes of sugar, sugar water and water were placed: in the glass with sugar water there was a folded piece of muslin and in the water a piece of cotton wool, so that if the insects fell in they could easily get out. At first honey was used as food, but as they could not extricate themselves from this it was soon given up.

Towards the end of the winter the percentage of maggots from the infected material that developed into flies seemed to increase. As this might possibly be caused by an injurious effect upon the development of the sexual organs of *Alysia* due to a too one-sided diet I tried to counteract it by adding a very small amount of bakers yeast to the sugar water, with doubtful success.

In each experimental cage stood a glass dish full of soil. At first, where the ♀♀ *Alysia* possibly had already laid eggs outside, a number of maggots of *Calliphora* in meat were put down. When, however, I discovered that the ♀♀ lived longer when they were not given the opportunity of laying eggs and that moreover the consequent increased urgency to oviposit could only be of advantage at the starting of fresh cultures of parasitized maggots, wasps and maggots were no longer put into the experimental cages together.

The experimental cages were kept at ordinary room temperature in the laboratory throughout the year.

The length of time that the imagines of *Alysia* remained alive in the conditions described above, corresponded better to the figures given by ALTSON (1920) and MYERS (1927) than to the much shorter period given by GRAHAM SMITH (1919). The number of days in my observations were somewhat less than the two former give. This must be attributed to the fact that the cultivation took place during the winter. ALTSON (p. 213) also stated that the length of life of the parasites was shorter from November to January and that this was apparently independent of the temperature at which the culture was kept. For ♂♂ the maximum length of life registered in my experiments was 23 days and for ♀♀ 28 days. The mean duration of life in captivity, however, calculated from 24 and 32 observations was 13.7 and 12.6 days respectively. The creatures proved to live longer at a low temperature (down to 10° C.). No regular observations were made, however, concerning temperature and humidity.

It would be a good thing to extend the observations, made so far, on the length of life of *Alysia* in captivity, accurately noting the temperature, humidity and duration and intensity of illumination. Only when this has been done can we know the possibilities for conveying the parasites to distant places, for use as biological control and how to keep them in good condition for stock-cultures.

To insure a constant supply of maggots of different ages I instituted a culture of *Calliphora erythrocephala* as well. Thus at any moment when a parasite came out, I was able to use certainly non-parasitized maggots in comparative experiments concerning infection. A number of imagines of *Calliphora* were placed in an experimental cage measuring 26 $\times$ 26 $\times$ 35 cm with walls of wire gauze, containing a dish of sugar and well saturated cotton wool, as well as petri dishes with pieces of raw beef, on which the flies could lay their eggs. Fresh pieces of meat were supplied as soon as the old ones became too dry, or when they had been sufficiently blown. On the meat I constantly found specimens sucking, that were not laying eggs, thus it apparently served as food as well. After eggs had been laid on them, the pieces of meat were transferred to covered glass vessels, half filled with earth mixed with peat

fibre, the latter making it easier to regulate the moisture. More meat was supplied when needed. Here the maggots developed further. It appeared that the moisture of the ground must not be too great, and especially that not too many maggots should be cultivated in each pot. The maximum proved to be not more than 50 on about 500 cc of soil. If these conditions were fulfilled, the pupation took place normally.

The pupae were transferred to a glass dish with soil in the experimental cage where the imagines were. As LOWNE (1892) stated in his extensive research on this species of fly, the ♂♂ were almost immediately ready to pair, while the ♀♀ on the contrary did not admit it till after about 2 weeks. After the culture had been continued for some time, the experimental cage contained imagines of such varying age that I could procure egg-deposits every day if desired.

BAER (1931) who made sterile cultures of maggots of *Calliphora* to use them for combating osteomyelitis and for keeping open wounds free of bacteria, gave as food for them a mixture of meat and liver with the addition of some yeast and for the full-grown flies a mixture of honey, yeast and water. When I tried his method, however, I found it to be too elaborate, as the food in the experimental cage had to be renewed every day, and as it does not keep, had to be prepared fresh each time. As my original method yielded good results, I adhered to it.

2. Parasitizing of *Calliphora* maggots in the Laboratory.

The parasitizing almost always took place under a petri dish which was placed upside down upon a piece of white filter paper. Beneath this a single or several *Alysia* ♀♀ and some maggots were brought together. After the maggots had been stung they were removed one at a time and the wasp was supplied with fresh maggots. On dark afternoons in the winter months the stinging sometimes occurred more easily if a lamp was switched on above the petri dish (50 watt at a distance of 40 cm). The infected maggots were further cultivated in meat laid upon a mixture of earth and peat fibre in closed glass dishes. The meat was kept damp and was removed when the maggots left it to pupate in the substratum.

The parasitizations in the laboratory had two purposes. Some

only purposed the further cultivation of *Alysia* for sustaining the supply of imagines and some to supply an answer to certain problems concerning the effect of particulars of either parasite or host on the result of the parasitism.

The first were intended to produce the largest possible number of parasites. To increase the possibility of successful parasitization maggots from different cultures and therefore of different ages were taken, while as parasitizing wasps several fertilized ♀ ♀ were chosen which seemed to be in good condition and that stung readily.

By these methods I was actually able, even in the winter months, to obtain a pretty constant supply of imagines of *Alysia manducator*, both ♀ ♀ and ♂ ♂. Nevertheless, the number of infections that lead to the coming out of *Alysia* was poor. The number of descendants that the ♀ ♀ produced under these artificial conditions, was greatly below the number which SALT (1932, p. 241—242) procured in his experiments with *Lucilia sericata* as host.

In the second category either several collections of maggots, differing from each other in one or more particular but each collection as far as possible uniform in itself, were attacked by a single parasite or one collection was divided into sections and each section was stung by a different wasp, the characteristics of which were known to be different.

With this carefully controlled parasitization the solution of various problems was begun.

α. ALTSON (1920, p. 213) stated that no research had been made upon the fertility of ♂ ♂ of *Alysia*, which had come from eggs laid by unfertilized ♀ ♀. To investigate this problem ♀ ♀ that had just come out were introduced to ♂ ♂ of parthenogenetic origin and after copulation had taken place, were given the opportunity of parasitizing a set of maggots. The cultures of these maggots at first yielded only ♂ ♂ of *Alysia* or specimens of *Calliphora*. It became evident that this was due entirely to unfavourable conditions at copulation or oviposition or development of the eggs, when from a set of maggots of this kind 1 ♂ and 4 ♀ ♀ of the parasite were obtained. This proved that, as might be expected, ♂ ♂ of *Alysia* of parthenogenetic origin are actually fertile.

β. When both parasite and host were cultivated for some time in the laboratory it seemed as if the procentual number

of maggots that developed into flies after they had been stung increased. It was therefore necessary to ascertain to what influence this was due. As even with the most minute observation it proved to be impossible to state whether at the moment that the parasite inserted the ovipositor in the maggot an egg was actually deposited (cf. p. 436) I endeavoured to get a better idea of it by carrying out different infections. At first I used the culture material that I had in hand, while later on I could compare it with specimens collected out of doors in the springtime. The following questions were asked:

β_1. Does the size of the maggot at the moment of parasitization effect the chance of a normal development of the parasite?

Although, neither in nature nor in the laboratory, a preference of *Alysia* for a special size of maggot could be detected, it might be possible that in parasitization in the laboratory maggots were presented to the wasp which differed from those chosen in nature. The possibility of a destruction of the parasite egg or larva by a direct action of the host (cf. BOESE, 1936) might be greatest in maggots of a particular size, but I could not observe any signs of it in my serial sections of parasitized material. Moreover it is not impossible that a maggot of a particular size might possess qualities that made it impossible for the parasite to oviposit. Difference in turgescency of the maggots or difference in the thickness or toughness of the cuticle might lead to no egg being deposited even when the ovipositor had been inserted.

To solve these questions, 30 maggots of 11 by 2 mm, 30 of 14 by 3 mm and 30 of 15 by 4 mm were taken from the laboratory cultures. Then 2 maggots of each size, that is 6 at a time, were introduced to one *Alysia* ♀ under a petri dish, and after they had been once stung were removed. This was repeated fifteen times until all 90 maggots has been attacked. The maggots were further cultivated in separate dishes, but under the same conditions, so as later to be able to compare the number of parasites which came out.

In this experiment, also, the parasite showed no preference for a particular size, but stung the maggot with which it first came into contact without any hesitation.

Similar experiments, in which only 2 sets where the maggots differed in size were exposed to one *Alysia* ♀, were repeated several times. The sets might in that case each consist of a larger number of maggots.

β_2. Does the age of the wasp at the moment of parasitization effect the probability of normal development of the parasite?

In dealing with this question I had to take into consideration the fact that all the stings of the parasite might not be successful. If, for instance, this should be the case with the first stings made by a ♀ that began to oviposit, it might have a great effect upon the cultures that I had made, as I often worked with wasps that had only just come out.

To investigate this possibility a fertilized *Alysia* ♀ which had not yet laid any eggs was given the opportunity on five successive days, to oviposit in 30 maggots of 13 by 3 mm from the laboratory cultures. In the intervals, the parasite was kept apart, supplied with food but not with maggots. The time in which the parasitization of the 30 maggots took place was 63 minutes, 25 minutes, 40 minutes, 79 minutes and 67 minutes respectively. On the last day the parasite moved uncertainly, with the hindmost pair of legs further apart than normal and was no longer able to crawl up the vertical rim of the petri dish. It stung repeatedly amiss and made tentative movements with its ovipositor.

Here again the maggots were cultivated further under uniform conditions. From the number of parasites which the various collections of maggots might yield, a difference might be shown between the first and last stung of a larger number of maggots parasitized on successive days.

This difference might also exist if a similar number were stung in a single day. In the experiments which gave rise to these questions such a large number of maggots were never parasitized on one day so that any possible abnormalities under such conditions were not further investigated.

β_3. Does the cultivation under unnatural conditions have a degenerating effect upon the parasite?

It has more than once been shown that an unfavourable effect upon the fertility of insects is produced by abnormalities of food, light, or other similar factors, to which cultivation in artificial conditions may give rise. COUSIN (1929) and SALT (1932) stated that in *Lucilia sericata* no eggs developed in the ovaries unless the food contained amongst other things a certain amount of meat, while SALT (1932) further maintained that in this species the eggs laid were for a great part sterile if the cultures were carried on in artificial light, even if the intensity of the light was high.

Although in my cultures of *Alysia manducator* no direct indications were found of such abnormalities, it was desirable to investigate the possibility of such, by a comparison of cultured material with specimens found in nature. For this purpose the following experiment was set up:

Of 100 maggots of 12 by 3 mm from a laboratory culture 50 were stung once by an *Alysia* ♀ caught outside and 50 by a ♀ that had hatched in the laboratory. Each set of maggots was cultivated further separately.

β_4. Does the parasitization of maggots of a collection of *Calliphora erythrocephala* cultivated from one generation to another under artificial conditions give a smaller chance of normal development of the parasite?

It might be that conditions such as food and other factors of the environment in the laboratory, would account for the differences which were discussed in reference to the exposing of maggots of various sizes (cf. β_1) and might result also in differences between maggots cultivated in the laboratory and those found upon the bait.

To investigate this further some ♀♀ of *Calliphora erythrocephala* were caught outside, who immediately began to deposit eggs. When the maggots that developed from these had reached the size of 12 by 3 mm an *Alysia* ♀ was given the opportunity to sting once a number of these alternately with a number of maggots of the same size from a laboratory culture which had been going for at least four months. This was continued until 50 maggots of each kind had been parasitized, after which they were further cultivated separately.

γ. In the experiments described elsewhere concerning the effect of parasitization upon the moment of pupation (cf. p. 429) some sets of once stung maggots were compared with twice stung specimens. This material was then further cultivated at first with the sole purpose of using the parasites which came out for the further infection of maggots. It proved, however, that the different number of stings effected the number of parasites hatching. On one occasion 35 once stung maggots yielded one full grown *Alysia* while on the other hand 35 twice stung maggots produced 10 specimens of the parasite. In other cases the difference was not so striking, but the matter seemed worth further investigation. A couple of readily stinging *Alysia* ♀♀ were introduced in the usual way to maggots of 13 by 3 mm until 90 had been

parasitized. Of these 90, 60 were then stung for a second time by the same ♀ ♀ and finally 30 of these were given a third sting. These collections were further cultivated separately, but under the same conditions.

In all the parasitizations described above, the percentage of flies produced in the further cultivation of the parasitized maggots was very high. The number of parasites that these cultures yielded was therefore too small to form a reliable basis for conclusions.

Unfortunately, I was forced to break off this series of experiments before being able to extend my data sufficiently to supply an answer to the interesting problems which they raised.

CHAPTER III

THE POSSIBILITY OF PARASITIZATION BY *ALYSIA MANDUCATOR* INDUCING AN EARLIER PUPATION OF THE HOST

1. Data from Literature.

When an insect is made use of as host by one or more parasites, the moment at which the transition to the following stage takes place may be different from the normal time at which it occurs in an unparasitized specimen of the same species. It is obvious that the effect of the developing parasite will be very evident at the transition from larva to pupa.

PANTEL (1913, p. 121—124) gave a number of instances of this effect. He described cases in which the normal specimen pupated earlier than the parasitized ones, while on the other hand, there were cases in which it was the parasitized ones which pupated first. He summarised these variations as follows (p. 123):

"Le ralentissement de l'ontogenèse semble être lié à un degré de l'affaiblissement parasitaire qui doit être atteint, mais non dépassé; l'influence doit être assez marquée pour empêcher l'organisme hospitalier de parvenir à l'état de développement externe et de maturité interne qui amène normalement la crise nymphale; mais si elle est trop accentuée elle pourra donner *avant l'heure* le signal de cette même crise."

This statement of the problems of parasitic influence upon the host, is given a very universal form, but on that account leads in my opinion too much to generalizations as to the effect upon the different hosts. Both the data furnished by later writers concerning the effect of *Alysia* upon the pupation of its various hosts and the results yielded by my own experiments, have convinced me that the question must be examined separately for every parasite and in relation to each host.

In *Alysia manducator* the case seems to me to be of a very complicated nature. Not only does *Alysia* make use of different hosts, each of which may respond in a different way to the parasitization, but considering that *Alysia* attacks maggots of varying size, the age of the host at the moment of attack may influence the matter. Moreover it may be important to know whether the hosts, in which *Alysia* hibernates, normally pass the winter as maggot or as pupa, a question which is not yet answered in regard to all species that are used as host (GRAHAM SMITH, 1916; DAVIES, 1930). In the latter case a pupation before the beginning of the winter will seldom reveal itself as being due to parasitism.

ALTSON (1920, p. 211) was the first to state that the host of *Alysia manducator* was stimulated to an earlier pupation by parasitization. But he did not mention what host was made use of and his experiments do not appear to me convincing (they will be further discussed in this chapter in the description of my own research). It is different, however, with the extensive research carried out by HOLDAWAY and EVANS (1930) and SALT (1932) on the effect of *Alysia* upon the pupation of *Lucilia sericata* Meig. Here there was no doubt of a hastened pupation, but when SALT says (1932, p. 243): "This, of course, is only a specific case of a well-known phenomenon (cf. PANTEL)", in my opinion he is too ready to apply to other species of host the conclusions obtained in regard to *Lucilia sericata*.

MYERS (1927, p. 225) gave instances in which the pupation after parasitization did not lead to the pupa coarctata, but where the pupa preserved the form and segmentation of the maggot, so that something more like a larva coarctata was formed. He worked chiefly upon *Calliphora erythrocephala* Meig. so I assume that it was here he observed it. I regard these cases as being due to abnormal and not to hastened pupation.

A second point, which is not directly connected with the problem under discussion, but which is of the utmost importance

to my further research, is the effect of an eventual accelerated pupation upon the possibility of superparasitism. ALTSON (1920) assumed that the maggot, immediately after being stung by *Alysia*, hastened to bury itself for pupation, but if the parasitized maggot were again successfully attacked by *Alysia* on its way to the protecting substratum, it would perish within 24 to 48 hours. This would make the chance of superparasitism very small and if, by exception, two or more parasite-eggs should be laid in the maggot, they would never be able to develop in nature. Let me state at once, however, that in my research, not only maggots which had been twice parasitized continued their development, but even those which had been stung more than five times achieved a complete pupation. GRAHAM SMITH (1919, p. 389) too mentioned the hatching of flies and parasites after the pupation of twice stung maggots. It is therefore of real value to study not only the development of parasites that are found singly in the host, but to collect data concerning the cases in which more than one parasite develops in the host and concerning the competition which is bound to arise between these parasites. Moreover these cases of superparasitism might effect the development of the host in some special way.

In the following paragraphs I shall discuss the causes which may give rise to an earlier pupation for instance of parasitized *Lucilia sericata*.

While PANTEL (1913) spoke of "un degré de l'affaiblissement parasitaire" as causing the behaviour he described of the host, ALTSON (1920) attributed it to "effective oviposition". On the other hand HOLDAWAY and EVANS (1930) remarked: "One hesitates to accept the idea that 'successful parasitism' by *Alysia manducator*, if this means the actual presence of an egg capable of development within the host, is the cause of the stimulated pupation in *Lucilia sericata*. It may be that the real cause is the secretion injected at the time of oviposition and which causes temporary paralysis of the host larva."

If we accept the theory of ALTSON (1920) and others, that the parasite egg practically remains further undeveloped till the histolysis of the tissues of the host in pupation, it seems incomprehensible that this egg should have so great an effect. It appeared to me, however, (cf. p. 477) that from an *Alysia* egg in *Calliphora erythrocephala* at the pupation of the host the parasite larva may have developed as far as the second larval stage at

least. In *Lucilia sericata*, also, the development of the parasite may be considerably advanced at the pupation of the host. I think it highly probable that although the parasite larva at this stage feeds on haemolymph only and has not yet damaged the tissues of the host to any great extent, its increase of size might be the cause of accelerated pupation. Whether the poison injected by the mother insect effects the pupation and, if so, in what way, should be studied in *Lucilia sericata* or another species in which hastened pupation has been proved, by a comparison of singly and repeatedly parasitized specimens.

2. My own experiments.

As I have said, ALTSON's experiments (1920, p. 233—235) did not convince me, particularly because there was no control of unparasitized material which had been subjected to the same treatment. I set up a number of similar experiments in which this objection was removed. Only *Calliphora erythrocephala* Meig. was used as a host, the supply being continuously cultivated here in the laboratory. As GRAHAM SMITH (1916) had come to the conclusion that this species hibernated principally as pupa and as this part of my research was carried out during the winter months, it was possible that the difference of behaviour of parasitized and non-parasitized specimens would not be very conspicuous. For this reason, besides collecting data concerning a possible acceleration of pupation due to parasitization, I made a special study of the behaviour of the maggot immediately, or very soon, after the oviposition, as in this period the possibility of superparasitism is terminated.

Experiment I.   Three cylindrical glass vessels with an internal diameter of 9.5 cm were filled with damp earth to a height of 12 cm. The earth was entirely covered by a layer of raw beef 2 cm thick. In a petri-dish I then allowed a fertilized *Alysia* ♀ to sting 20 maggots twice and 20 maggots a single time. The maggots were 11 mm long and at the height of the clearly visible crop 2 mm thick. Immediately after the parasitization a twice stung maggot was put into glass 1 and a once stung into glass 2 in succession while an unparasitized maggot of the same size was placed in glass 3. All the glasses were kept in the daylight. After five days they were carefully ladled out. The maggot, *in all three glasses* were then found comparatively evenly distributed over the whole available depth of the soil. There appeared to be no partiality for a special depth.

This experiment was repeated, using raw ox liver as food. Again maggots were used of 11 by 2 mm and a fertilized *Alysia* ♀, while now 35 maggots were placed in each glass. They were examined after 7 days (By the conditions

in my other cultures I always was able to judge the presumable time of pupation). Here too the maggots were evenly distributed in all the glasses.

The principal results of these experiments are collected in table 1.

Table 1.

		Stung twice	Stung once	Unstung
After 5 days	Number	20	20	20
	Maggots	8	1	3 + 1 †
	Pupated	12	19	16
After 7 days	Number	35	35	35
	Maggots	6 + 2 †	3	0 + 2 †
	Pupated	27	29	31
	Missing	0	3	2

Experiment II. In a dish of about 20 cm internal diameter a layer of earth was entirely covered with thin slices of ox liver. 30 maggots of 11 by 2 mm were placed upon the liver. A number remained on the surface to feed. A fertilized *Alysia* ♀ was then allowed to attack the maggots.

a. A maggot feeding on the surface was stung. It became paralised. At the first movement after the paralysis the maggot was stung for the second time which again produced paralysis, but for a much shorter period. After recovering from the second paralysis the maggot crawled about the surface of the liver for 1 minute and 35 seconds, after which it disappeared down a cleft.

b. A second maggot feeding on the surface was stung. Paralysis followed. When it began to creep again the *Alysia* ♀ was prevented from approaching it. The maggot began to feed again. After 3 minutes the *Alysia* ♀ was admitted to the maggot and stung it a second time. This was followed again by paralysis, of shorter duration than the first. Having recovered from this paralysis the maggot continued its feeding in the normal way.

(After the experiments described above had been carried out the same maggots were all stung once by the same *Alysia* ♀. One specimen was so badly wounded that its internal organs burst out. The remaining 29 larvae, after this parasitization were removed to a vessel containing earth covered with liver. 1 h 30' later 14 maggots were found in the earth, but 15 were still in the food).

Experiment III. Two exactly similar glass vessels of 4.7 cm diameter internal measurement and 6 cm height were filled with a layer of earth covered by a layer of raw ox liver. Simultaneously a number of maggots stung once by a fertilized ♀ were placed in one and in the other the same number of unparasitized maggots of the same size. The two vessels were kept under the same conditions and the parasitized material was alternately placed in the one and in the other. After a time the position of the maggots was examined. These manipulations were repeated several times with sets of maggots of each given size to collect a sufficient number of data.

The number of maggots that were put into each of the vessels at a time depended upon the rapidity with which the *Alysia* ♀ attacked. If the parasitization occupied a comparatively long time, I had to be content with smaller sets of maggots per time, as otherwise there might be too great a time-difference between the moment at which the first and the last maggot was placed in the experimental jar, which would give rise to difficulties when comparing the data collected.

The times given for the controls were calculated from the moment at which the last maggot of a set was placed in the experimental vessel. Some of the maggots, therefore, were a little longer in the jar than the time stated, but in both jars this was exactly the same.

a. Size of the maggots 6 × 1 mm. As these small maggots crept slowly and had only little strength for penetrating the liver, I used 1 cm liver upon 2.5 cm earth. Moreover a slightly longer time was allowed to elapse between introducing the maggots into the experimental vessel and controlling the contents viz. 15 min. The parasitizing was done by several *Alysia* ♀ ♀. Up to 20 maggots were exposed to the parasitizing ♀ ♀ in a petri dish. When 10 maggots had been attacked once the Braconids were removed. The paralysis of these maggots was very short. The parasitized maggots were placed in one vessel and the unparasitized in the other.

6 series were carried out in this way, each comprising 10 parasitized and 10 unparasitized specimens.

b. Size of the maggots 11 by 2 mm. The vessels contained 1.5 cm liver upon 4 cm earth. The parasitization took place by 1 fertilized *Alysia* ♀. 5 to 6 maggots were placed under each of two upturned petri dishes standing on white paper and under one of these the *Alysia* ♀ was introduced. Each time that a maggot was stung this maggot and one unparasitized maggot from the other dish, were placed in the two experimental vessels. Control after 12 to 13 minutes.

± 10 series were carried out, each comprising 5 to 6 parasitized and the same number of unparasitized specimens.

c. Size of the maggots 14 by 3 mm. In this experiment, which was the first to be carried out, only one experimental vessel was used, containing 2 cm liver upon 4 cm earth. The parasitization by one fertilized *Alysia* ♀ took place on the surface of the liver. The position was observed alternately of 3 parasitized and 3 unparasitized maggots after 12 minutes.

± 20 series were carried out, comprising 54 parasitized specimens in total and alternating with ± 20 series comprising 58 unparasitized specimens in total.

d. Size of the maggots 14 by 4 mm. Maggots which were yellowish white, no longer feeding and of which the crop was no longer visible externally. Two experimental vessels were used, containing 1.5 cm liver upon 4 cm earth. 5 maggots were parasitized each time by a fertilized *Alysia* ♀ exactly as described in *b.* As the paralysis of these specimens lasted for some time (2 to 3 min) the controls were made 15 minutes after placing the last maggot of a set in the experimental vessel.

12 series were carried out in this way, each comprising 5 parasitized and 5 unparasitized specimens.

The results of experiments *a–d* are given in table 2.

Table 2.

Size and Stage of maggots		Total num-ber	Time of each experi-ment	Non-parasitized			Parasitized		
				in food	in earth	(mean depth)	in food	in earth	(mean depth)
6 × 1 mm	feeding	120	15′	60	0	—	60	0	—
11 × 2 mm	feeding	120	12–13′	51	9	(2.0)	48	12	(2.4)
14 × 3 mm	still just feeding	112	12′	40	18	(2.0)	37	17	(1.4)
14 × 4 mm	full-grown, not feeding	121	15′	12	49	(1.4)	13	47	(1.7)

3. Discussion of the results.

Experiment I. In this experiment a comparatively small number of hosts were used, which naturally decreases the value of the results, but it should be noticed that there were no signs of an accelerated pupation of the parasitized specimens in these experiments. I do not consider that the effect which ALTSON (1920) attributes to the parasite is by any means proved, and after comparing the figures obtained here I am inclined rather to conclude that in *Calliphora erythrocephala* no acceleration of pupation occurs after parasitization.

Further, on the grounds of my experiments, I do not think that in *Calliphora erythrocephala* the poison injected by *Alysia* before depositing its eggs has the effect attributed to it by HOLDAWAY and EVANS (1930). If this effect existed, the twice stung maggots ought to be inclined to an earlier pupation than the once stung specimens, as they would have received a larger amount of the poison. The controls showed, on the contrary, that a larger number of the twice stung maggots remained in the larval stage.

(In the last series a few specimens were missing in the controls. I think it probable that these perished as maggots and were not traceable after they had dried up as after a short time only cuticle and endodermis remain of dead maggots).

Experiment II. The arrangement of this experiment was not very satisfactory, because both the specimens which were not parasitized on the surface of the food and those which dis-

appeared into the liver after being stung could not be followed further. Nevertheless the behaviour of the parasitized maggots in this experiment showed plainly that a maggot of *Calliphora erythrocephala* does not invariably burrow after parasitization, as ALTSON asserts. In the following experiment, also, numerous data confirming this were collected all showing that the possibility of super-parasitism is not excluded.

Experiment III. During the earlier experiments I had seen indications that both parasitized and un-parasitized maggots of *Calliphora erythrocephala* were inclined to burrow under special circumstances. The object of this experiment was to trace the cause of this. As I thought it probable that the state of nutrition of the maggot played an important part here I used series of varying ages of which the maggots were as far as possible of an uniform size. The oldest series consisted of maggots from a culture where they had just abandoned the food to begin the period of rest previous to pupation. In nature it will be very rare for a maggot to be parasitized at this stage as it is then buried in the earth. Nevertheless, I considered it essential to include maggots in this stage so as to make my series of experiments complete.

As I have said, there was always an interval of 12 to 15 minutes between the parasitization and the examination of the contents of the experimental vessel. I chose this interval of time because it gives the best chance of the creature being again noticed by the *Alysia* ♀ and therefore the greatest likelihood of superparasitism. A small number of experiments with maggots of various ages, and in which the controls were made 1 hour after the parasitization yielded results corresponding entirely to those in which the controls were made after a shorter time.

A study of Table 2 shows at once that in none of the collections used was there any difference at all in the behaviour of parasitized and un-parasitized maggots. On the other hand, the number of maggots that leave the food and seek the substratum, increases regularly with age in both cases.

This makes it certain that the premature burrowing of maggots of *Calliphora erythrocephala* is not caused by parasitization but is decisively influenced by the stage they have reached and their condition of nutriment.

CHAPTER IV

THE DEVELOPMENT OF *ALYSIA MANDUCATOR* IN *CALLIPHORA ERYTHROCEPHALA*

A. Methods of research.

In studying the development of a parasite in its host, it is usually the total duration of the development which claims most interest. This is especially so in the case of insects of economic significance because this is the point of greatest practical value.

And yet it must always be of importance to collect as much data as possible about every stage that the parasite passes through. From the point of view of pure science this method is a matter of course, but from the practical point of view, also, a thorough examination of the whole problem is always desirable. Not only does it give more certainty as to whether certain methods of control may be useful or not, but moreover numerous facts may come to light which, although they may at first seem insignificant, at any moment may prove to be of great value in estimating the relation between parasite and host.

A logical method of reaching this goal is to take a large collection of specimens of the host that have been parasitized on the same day and fix them at regular intervals of time. By tracing the various stages of the parasite in this fixed material we get a picture of its development. As a rule this can be done by dissection of the host, but that alone is not sufficient if we want to ascertain with accuracy the position of the parasite at the various stages, to see if any movements have been made by the creature during its development. Moreover, while dissecting little trace will be found of any contact that may have been formed between the parasite and the tissues of the host. Such questions can only be settled by making microscopic preparations of the material to be examined. This method has moreover the great advantage that the development of the internal organs of the parasite can also be studied in every stage. As I constantly kept this possibility in view I always fixed my material in such a way that it could be used for microscopic preparations. This method, especially in regard to insect material, was naturally somewhat elaborate and took much time. Thus it was desirable to find a method for ascertaining with certainty that parasitizations which were

made, had really been successful. I tried in a variety of ways to test this.

1. Preliminary Research.

a. *Direct observation of the oviposition.*

The success of a parasitization could of course be best confirmed, if during the time that an *Alysia* ♀ kept its ovipositor inserted in the maggot of *Calliphora* it could be ascertained that an egg was actually deposited. To see if this were possible a large number of parasitizations were carried out under a Leitz binocular magnifier (magnifying power × 32). The instrument contained a lamp which assured the most favourable lighting of the object. Parasite and host were brought together in the usual way under an upturned petri dish, which was placed upon a card upon which a light coloured piece of paper was pasted, and slid onto the stage of the magnifier. The card could be moved by the left hand so that the parasite was continuously in the field of vision, while with the right hand the focussing of the instrument was regulated. Although under these conditions certainly 250 maggots were parasitized, it was seldom possible to see the point of the ovipositor disappear into the maggot, as it only emerges from the sheath at the very last moment before rapid insertion. Moreover the maggots crawled by preference along the raised rim of the petri dish and were therefore most often attacked there, and owing to the thickness and unevenness of the glass could not be sharply focussed. It also frequently occurred that during the oviposition the parasite sat on top of the maggot so that the ovipositor could not be seen through the wings.

To avoid these difficulties the upturned petri dish was replaced by a watch glass of 7 cm diameter. The maggots here also crawled as far as possible along the periphery and were to some extent caught under the rim of the glass. Not only did this reduce their struggles on receiving the sting, making the focussing much easier, but it had the great advantage that the wasp now usually attacked the maggot from the side so that the whole process could be followed properly. A great number of maggots were stung in this way. At the same time the conditions seemed highly favourable, especially when the ovipositor was placed in a completely transparent part of the maggot, or when the sting struck it tangentially, so that the point of the ovipositor

came through to close under the cuticle. Moreover now, especially when the ♀ had stung a large number of maggots and fatigue had caused some deviations from the normal process, the point of the ovipositor could often be examined just before it pierced. Nevertheless the actual depositing of an egg was never observed during the time that the ovipositor remained inserted, neither was any egg seen passing down the ovipositor at the last moment before it disappeared into the victim.

The further culture of a number of maggots used in these experiments showed that at any rate a part of the infections must have been successful which, however, had not been ascertained at the oviposition. It is true that I several times saw a clear, tough drop of liquid slide along the dorsal side of the ovipositor. Three times it happened that an exhausted ♀, after being thrown off by the maggot attacked, was making vague movements with the ovipositor, unexpectedly deposited an egg upon the paper beneath it.

b. *Making the host transparent.*

This method was tried, as it might prove a quicker way of examining the development of the parasite. But as a great many maggots were needed for the experiments described in these paragraphs, and as at that time I had not got a great number of infected maggots at my disposal, I worked for this preliminary purpose on unparasitized material. It was safe to assume that in cases where a good view could be obtained of the internal organs of the host, the parasite would not be able to escape attention.

The success achieved was very different, according as I worked on young, still feeding maggots, or older ones that had already entered the period of rest. The latter are of such a compact consistency that, even if I succeeded in making them transparent, I could never perceive a differentiation of the organs. With younger maggots, on the other hand, I was very successful.

After the creatures had been fixed, they were dehydrated and put into xylene. Very young specimens became transparent in this liquid. The fixation took place at a temparature of 60—70° C. This had the advantage that the creatures were stretched to their full length. At first they were fixed in alcohol 80 %, but then it sometimes happened that for some mysterious

reason, all the tissues became black, so that the object was useless. After that formaldehyd 40 % was used. The objects then were put into formaldehyd 4 % for some time and finally, when they had been dehydrated, they were placed in xylene. In somewhat older specimens the process of making them transparent ran quicker and more intensively if after the xylene they were placed in tetralin (DRAHN, 1922). Sometimes a preliminary treatment in diaphanol (a solution of Cl O$_2$ in acetic acid, SCHULZE, 1922) seemed desirable. After remaining 2 days in diaphanol, followed by passing through the alcohols, objects that did not become sufficiently transparent through the first treatment could be made suitable in tetralin for the examination of their internal organs in less than 24 hours.

In studying pupated material these methods proved to be unsuitable. But by de-pigmentation with diaphanol it could easily be seen if the parasite had already done serious damage to the tissues of the host and whether its development had proceeded to the last larval stage.

c. Simplified method of preparing out the parasite.

In the preliminary research the method of preparing out, which later became one of the regular ways of examination, was applied in a simplified form, to give a rapid survey of the development of the parasite. The fixation took place in boiling water acidified with acetic acid to increase the penetrative power. It was not necessary to leave the objects in this for more than 3 minutes. Although this fixation was a fairly drastic method, it seemed to cause no serious dislocations in the tissues by contortion, so that it might be assumed that the correct position of the parasite could be ascertained sufficiently for preliminary purposes. The maggots treated in this way at a high temperature were again remarkably well stretched to full length.

In this method, also, a marked difference was found between maggots of varying age. Preparing out of the parasites was most successful with young hosts because here, even after fixation, the internal organs were distinguished from each other and from the parasite by a difference of colour, so that there was not much chance of the parasite being overlooked. In older maggots, which had already entered the stage of rest, the parasite was no longer in contrast with the organs of the host which were of a uniform white colour.

In maggots which would probably contain the parasite as an egg, these eggs were difficult to find in the coagulated tissues of the host, while in treating pupated material of the host the larva of the parasite was often damaged in preparing it out from the compact contents of the boiled pupa. In tracing parasites which were in the first larval stages in younger maggots, however, this method yielded satisfactory results.

Although the two last methods were useful to ascertain quickly whether at any rate some of the parasitizations carried out for the supply of infected research material had been successful, I did not take them into regular use. This was chiefly because I observed that in July and August 1939 the parasitizations carried out by ♀ ♀ of *Alysia* caught in nature had extremely good results. This enabled me to obtain for my investigations infected sets of *Calliphora* in which practically every parasitization had been successful. It was only extremely seldom that in a more minute examination of these maggots and pupae no parasite was present, so that an orientating preliminary research was unnecessary. The material was made ready at once either for the finally chosen method of preparing out or for making serial sections, according to methods which will be described later.

2. Final methods of research.

a. Microtechnique.

The maggots of *Calliphora erythrocephala* proved to be very awkward material, both for the preliminary work such as stretching to full length and fixing and for embedding and cutting, while the pupae, owing to the hard and thick chitinous wall, required very careful treatment. Although the puparium could be slit to allow the chemicals to penetrate more easily, without doing much harm to the pupa, it was impossible to do this with the maggots. The young maggot, especially, is a moisture holding, turgescent object, the internal organs of which bulge out at the slightest injury to the cuticle. Of course this would be disastrous, so no means could be used for helping the various liquids to penetrate more effectively and rapidly. Both in fixing and embedding the imperfect penetration gave rise to difficulties.

As first fixative FREILING's mixture was used (1932), with which satisfactory results had been obtained in larvae of insects.

I allowed the fixative to operate for the originally prescribed length of time needed for the mixture to cool down from 60° C. to room temperature as well as a longer period, up to 24 hours, after which the object was placed in 80 % alcohol. But after this treatment the object too often shrivelled, and moreover, as in making them transparent, the objects became black from the alcohol 80 % and were useless for further manipulations. I noticed by accident that this did not take place if alcohol 96 % was used instead of 80 %. The least shrivelled were some very young maggots which were subsequently subjected to a short treatment with diaphanol so as to make the chitine more easy to cut and finally after dehydration were embedded in paraffin with xylene as a go-between. But in this process the paraffin penetrated so imperfectly, that serious damage was done to the tissues in cutting and I could only make one even moderately good series of one single object. As might be expected, the sectioning of older maggots was even worse.

It was rather better when the embedding was done by PÉTERFI's method (1921). Here the objects are not placed in paraffin via xylene, but from alcohol 96 % in absolute alcohol and then in a solution of 10 gr desiccated celloidin in 1000 cc methyl benzoate and then via benzene in paraffin. This yielded a good series of a maggot which had been parasitized 4 days after hatching and had been fixed 7 days after that. This method, however, did not always lead to satisfactory results in my case, as became evident when a comparison was made with an object that had hatched on the same day, was parasitized a day earlier, but fixed at the same age; thus as far as development went it was almost exactly the same as the former specimen and was treated in the same way, but it was quite impossible to section it. A difficulty which frequently arose was that air got into the objects, which it was practically impossible to eliminate with a vacuum pump. The specimen was then insufficiently saturated by the paraffin and was apt to tear when it was cut. In ROMEIS's description (1932, p. 109) of PÉTERFI's method the advice given seems to me unsound. He recommends letting the specimen drip well out after being taken out of the methyl benzoate — celloidin solution before placing it in the benzene, so as to prevent drops of celloidin which are difficult to cut, being precipitated. But by treating it in this way there is great chance that air will get in.

In looking about for another fixative, I noticed a communication by BAUMGÄRTNER (1928) who, after trying a number of recipes, at last got good results from a method described by ZANDER (1921) with water of 80° C. Although his reproductions of transverse and longitudinal sections through the head of the bee (he did not state how thick the sections were) were very beautiful, I did not follow his example, as I considered that with maggots, where the tissues are so much looser, such a high temperature would be likely to cause contortion.

Guided by the good microtechnic results obtained by the school of KÜHN, especially the research made by BLAUSTEIN (1936) on the metamorphosis of *Ephestia Kühniella* Zeller, I tried the method used by this author. After fixing with Bouin's fixing mixture with ALLEN's modification (1926) followed embedding according to PÉTERFI's method. This process, however, proved to be unsuitable for the treatment of maggots. The objects which had come well through the preliminary treatment showed gas-bubbles when embedded and when these were pumped out they were left almost always practically squashed flat.

The most important thing was to find a fixative that completely avoided shrinkage, as this was the only way of being certain that the position of an eventual parasite inside the host had not been effected by the treatment. For this purpose 3 identical sets, consisting of both young and old maggots, were treated with the fixing mixtures of Carnoy and of Freiling and with 4% formaldehyd respectively. The best results were obtained with CARNOY's method (1897). It is true that now and then a small tear was found in the cuticle of the maggot, but almost always far in front, in the sixth and seventh segment, causing the crop to stick out, but hardly any other dislocations. Moreover the tendency to tear was greatly disminished if the mixture was prepared with acetic acid 30 % instead of acetic acid glacial, while the maggots remained beautifully turgescent. The fixing mixture was always freshly prepared immediately before use.

As the maggots when they were placed direct in the fixative sometimes drew in their heads, they were first narcotized in the fumes of ethyl acetate. It appeared that if they were subjected to the fumes for at least 2 hours this difficulty was removed if care was taken to produce the insensibility gradually. Moreover there was a chance then of the stigmata remaining open, which had

the further advantage that the chemicals could penetrate better and more quickly.

The last and unsolved difficulty occurred when a few specimens which had displayed no abnormalities during the whole preparation with fixative, alcohol, diaphanol, alcohol, Péterfi, benzene and even benzene-paraffin, on being placed in liquid paraffin brought to the right temperature, suddenly became compressed and assumed a straw coloured yellow exterior.

The embedding method was therefore once more changed. After the complete method according to PÉTERFI had been so far modified that no absolute alcohol was used, the change from absolute alcohol to methyl benzoate being considered injurious by WETZEL (1931), I finally adopted the methyl benzoate embedding described by him.

The treatment of pupated material was exactly the same as that applied to maggots, except that the narcotizing with ethyl acetate was naturally left out. At the fixation the puparia were cut at each extremity with a razor. If they remained floating in the fixing mixture, the air contained in them was evacuated by a vacuum pump, which always took place without difficulties and in a short time.

I may thus state briefly the method finally adopted for preparing my material for cutting sections:

a. Narcotizing (of maggots) in the fumes of ethyl acetate for at least 2 hours.
b. Fixation in diluted Carnoy's mixture (i.e. with acetic acid 30 % instead of acetic acid glacial) for about 18 hours.
c. 96 % alcohol for about 24 hours, which was renewed at least once.
d. 80 % alcohol for about 24 hours, which was renewed at least once.
e. Diaphanol, until the outside of the objects had become quite soft.
f. 80 % alcohol, renewed until the liquid was quite free of acid.
g. 96 % alcohol.
h. A mixture of 1 part methyl benzoate and 2 parts 96 % alcohol.
i. A mixture of 1 part methyl benzoate and 1 part 96 % alcohol.
j. A mixture of 2 parts methyl benzoate and 1 part 96 % alcohol.

k.   Methyl benzoate I.

l.   Methyl benzoate II.

m.   Methyl benzoate III. The time necessary to pass from *h.* to *m.* depended chiefly upon the moment at which the objects sank, but it was never less than from 24 to 48 hours.

n.   Embedding in paraffin of melting-point 65° C.

After this treatment the material was cut with a microtome, in which the entire object had to be made into serial sections, as the preliminary research had shown that the parasite was not bound to one particular place. The sections were nearly always of 5*μ* thickness. Both longitudinal and transverse sections were made. In the latter tearing of the tissues took place much less often and sometimes not at all.

Various methods were tried for staining the sections. The most satisfactory results were obtained by staining with Heidenhain's iron haematoxylin, while MALLORY's method (1900, p.15) also proved effective, especially when the dye was used in a diluted solution. The application of Heidenhain's iron haematoxylin followed by counter-staining with eosin and even more so of Ehrlich's haematoxylin with counter-staining of eosin proved to be unsuitable for my material. As staining with Heidenhain's iron haematoxylin gave the best results, and as in case anything should go wrong the most extensive data were available upon this method, I used it for practically all my material. The staining lasted for about 18 hours, after which the usual differentiations with iron alum followed.

In spite of all precautions, however, sometimes, especially in serial sections of older maggots, it was seen that shrinkage and tearing of the object had taken place. Luckily this evil was not of such an extent as to seriously hinder the further examination of the specimen. In older larvae of the parasite it was sometimes found that the mid-intestine, the contents of which had gradually assumed a more solid consistency, had not absorbed the paraffin, in which case the tearing, even in sections of great thickness, was considerable.

As I could only tell the direction of the sections with regard to the host and the position of the parasite was quite arbitrary, it sometimes happened that microtechnically successful sections did not yield the desired result because the parasite was struck at an unsuitable angle. A sufficient number of each set were

always fixed so as to have a reserve to fall back upon in such cases.

(After a portion of the material had remained unused for a time, owing to the research being temporarily broken off, the sets of parasitized specimens fixed at 144 h and 165 h 30 m after oviposition proved to have dried up so as to be useless. As the material fixed at 90 h 45 m after oviposition had not yielded many results, sections were made in series 11c, 12c and 13c for studying the internal organs of larvae of these 3 ages. Larvae of the desired ages which had been prepared out were embedded and treated in the way described above).

b.   Preparing out the parasite from the host.

When a set of parasitized maggots of a certain age had been fixed, only one half of them was treated further for embedding, while the other half was reserved to be used as material from which the parasite could be prepared out. Thus it was possible to compare each larva in the serial sections of the host with specimens of the same age which had been prepared out whole from the host.

Preparing out took place under the Leitz binocular magnifier (magnifying power × 32) in a petri dish containing distilled water. The objects were taken from the alcohol 80 % in which they were kept after fixation by CARNOY's method, and after being placed for a short time in alcohol 60 % and then in 30 % were put into the petri dish. A small quantity of alcoholic solution of cochineal was added to the distilled water at the moment when the object to be examined was placed in it, and gradually stained the tissues during the process of preparing out.

Staining with a solution of cochineal, being familiar as staining for egg yolks, was originally chosen when preparing out the parasite eggs in the hope that the egg looked for would be stained more darkly than the surrounding tissues. As it turned out, however, the egg remained white because the chorion almost entirely prevented the stain from penetrating into it, but the organs of the maggot took up the stain pretty quickly. Although the original purpose was therefore not attained, the method was still pursued and also applied in the more mature stages of the host, because the different rapidity at which the various tissues took up the stain gave a differentiation that was extremely

useful in the dissection. But if the dye according to Romeis's recipe (1932, p. 187) was used undiluted, the colouration took place much too quickly and all the tissues were equally red in a short time, and the useful differentiation was lost. So I diluted it further and obtained the best results when only 5 drops of the solution of cochineal were added each time to the about 12 cc distilled water that the petri dish contained. It proved that tap water must never be used in this manipulation as if it was, even with this very small amount of dye, the maggot rapidly turned dark red and then black (the Leiden tap water contains a lot of Ca).

When a parasite was to be prepared out from a maggot, independent of the stage of development the parasite might have reached, the cuticle of the maggot was carefully cut on opposite sides with a razor along its whole length. If necessary the cuticle was further loosened with fine pincet scissors and then prepared with teasing and straight needles. With these the peripheral bundles of muscles were then removed after which the examination of the deeper tissues was made.

Preparing out from pupae was also done entirely with needles, after the substantial chitinous wall of the puparium had been removed entirely or as much as possible, by means of tangential sections with a razor.

As it proved that even in cases when the ovipositor of the parasitizing ♀ had only been inserted in the maggot for such a short time that a deposition seemed improbable, an egg was present all the same, each specimen had to be examined to see whether it contained more than one egg. Even when a parasite was discovered at the beginning of a dissection of a maggot or pupa of *Calliphora* the host was carefully examined further to see if any more were to be found.

B. Data concerning the development of *Alysia manducator*.

1. Data from Literature.

When the larva of *Calliphora erythrocephala* is parasitized by *Alysia manducator*, its development is not immediately arrested. It can go on feeding just like an unparasitized larva, then goes through a period of rest in the ground and finally proceeds to pupate in the normal way.

ALTSON (1920) assumed that a parasitized maggot would pupate sooner, but my research did not confirm this, at any rate not for *Calliphora erythrocephala*. My views on the matter are stated in Chapter III, p. 432 and will not here be further discussed. For it remains a fact that the metamorphosis of the host might have a great influence upon the development of the parasite, even if the stages of the host followed one another at an increased rate.

In his extensive treatment of the stages of development of *Alysia manducator* in *Calliphora* ALTSON gave considerable weight to this fact. According to him, the development of the parasite is accelerated particularly at the moment at which the tissues of the host are in histolysis for the formation of the pupa. Previous to this the parasite would almost always be present as an egg only. Very occasionally the egg would hatch in the maggot, which would be evinced by abnormalities in the pupation of the host. The puparium would then assume a contorted and shrivelled appearance from the damage done to the muscular tissues of the maggot. The further development of the parasite, the moulting to the second larval stage and the following ones would take place exclusively in the pupa of the host. Finally, ALTSON observed in dissection, that when the puparium had been eaten out entirely the parasite was in the last larval stage, the duration of which varied from a few days to several months. The parasite now filled up the puparium entirely and was surrounded either by the wall of the puparium only or by the cuticle of the nymph of the host as well, within which the cocoon was spun after a certain time.

MYERS (1927) agreed with ALTSON. He stated that the presence of *Alysia* as egg or as young larva before the pupation of the host, was difficult to demonstrate by "gross dissection", but added: "Yet three or four days after pupation, under September conditions, it is rare to find anything in the puparium but a fullgrown *Alysia*-larva, with occasional traces of the victim's tissue at one or other end of the case. Usually the inner surface of the puparium is polished clean". The period of feeding of the parasite would terminate 10 days after the deposition of the egg. He also stated that the spinning of the cocoon often only took place some weeks after the pupation of the maggot.

A communication from MORGAN (1929) in which he says that the egg of *Alysia* came out 2 or 3 days after the oviposition

and that the length of time between the oviposition and the pupation of the host varied from 1 to 6 days, would lead us to suppose that he did not consider a first development of the parasite within the maggot of the host to be impossible. He did not pursue this subject, however, and quoted ALTSON and MYERS principally on the development of the parasite.

2. Entries of my own observations in preparing out.

For indicating the segments of the host I followed LOWNE (1890-92, I, p. 33-35). As illustration I have reproduced one of his plates, see figure 1.

The numbering of the segments of the host, especially in these entries, is therefore from front to back, so that the first externally visible segment is marked as "segment IV" and the ventral part of the anal segment as "segment XV".

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
8		22 h	1 *egg* attached to cuticle, high dorso-lateral in segment XI, embedded superficially in the muscular layer.
,,		,,	1 *egg* come loose in preparation (presumably attached to cuticle in segment XI, place of attachment (?)).
,,	maggots feeding (size at parasitization: 11–12 × 2–3 mm)	,,	1 *egg* attached to cuticle, lateral left in segment VI, free point sticking upwards almost at right angles to longitudinal axis of maggot.
,,		,,	1 *egg* attached to cuticle, lateral right in segment IX, free point sticking out sideways and forewards, piercing muscular layer.
,,		,,	1 *egg* attached to cuticle, dorso-lateral right in segment XI, directed backwards, embedded in muscular tissue.
,,		,,	1 *egg* attached to cuticle, dorso-lateral right at border of segments VIII and IX, lying along transversal muscle with free point downwards and backwards against the upper surface of muscular tissue.

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
9		45 h 35 m	1 *egg* attached to cuticle, dorsal in segment XI, directed backwards with free point piercing muscular layer.
,,		,,	1 *egg* attached to cuticle, ventral in segment VII, directed upwards and backwards, not sticking through muscular layer.
,,		,,	1 *egg* attached to cuticle, lateral left in anal segment (attached close beside spiraculum posterius), sloping upwards.
,,		,,	1 *egg* attached to cuticle, lateral somewhat ventral in segment XIII, sticking out backwards parallel to longitudinal axis of maggot, completely embedded in muscular layer.
,,		,,	1 *egg* muscular tissue, dorso-lateral in segment X (no contact with cuticle visible, completely surrounded by tissue).
,,		,,	1 *egg* attached to cuticle, ventral in anal segment (XV), sticks forwards into the tissues.
10		70 h	1 *egg* unattached in muscular tissue, dorso-lateral in segment IX, in which embryo is visible, with head towards tubercle of egg. Chorion not completely filled.
,,		,,	1 *egg* unattached in muscular tissue, lateral in segment X, embryo visible; embryo fills up chorion completely.
,,		,,	1 *egg* unattached in muscular tissue, lateral in segment XI, embryo visible, with head towards tubercle of egg.
,,		,,	1 *egg* unattached in fatty tissue against right tracheal main trunk in about segment XI. Embryo clearly visible.
,,		,,	1 *egg* unattached in muscular tissue, ventro-lateral in segment VIII, in which embryo is clearly visible.
,,		,,	1 *egg* attached to cuticle, dorsal in segment IX, lies parallel to longitudinal axis of maggot with free point forwards. Embryo indistinct in this egg.

(For set 9, in the "State of the host at fixation" column, running vertically: maggots feeding (size at parasitization: 11–12 × 2–3 mm).)

(For set 10, in the "State of the host at fixation" column, running vertically: maggots in rest period (size at parasitization: 11–12 × 2–3 mm, feeding afterwards for 48 h).)

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
11		90 h 45 m	1 *larva* in the fatty body near the centre of the maggot, close in front of curves of intestine (viz. about segment XI). Larva slender with tail-like abdominal appendage. Probably head, 11 segments, tail. Length: 0.85 mm + tail 0.15 mm. Thickness about 0.18 mm. Mandibles pigmented brown. Curved to horseshoe shape, lying in plane at right angles to longitudinal axis of maggot. Only contact with fatty tissue seems to be at the head.
,,		,,	1 *larva* firmly embedded in fatty tissue, just behind the imaginal discs (i.e. behind in segment VII). Larva short and compressed with fairly long tail. Length: 0.5 mm + tail 0.2 mm. Thickness 0.22 mm. Headcapsule larger in proportion than in former specimen. Along its whole length in close contact with fatty tissue.
,,		,,	1 *larva* unattached between lobes of fatty body and Malpighian tubes near the centre of maggot in segment XI. Lying in plane at right angles to longitudinal axis of maggot. Larva slender and clearly consisting of head, 11 segments, tail. Length: 1.1 mm + tail 0.2 mm. Thickness 0.18 mm.
,,		,,	1 *larva* unattached in the loose ventral tissues of the anal segment (XV). Damaged in preparing out, so that there are no reliable data concerning length, etc.
,,		,,	1 *larva* unattached, just inside muscular layer, lateral in segment XII or XIII. Larva short and compressed, entirely surrounded by a layer of tissue. This may contain the burst chorion. After removal of this layer length is 0.7 mm + tail 0.15 mm. Close against each other are to be seen head, 11 segments, tail.
,,		,,	1 *larva* against wing-disc in segment VI. Tail attached to disc, head protrudes further forward. Fairly large larva of the slender type with long tail. (Specimen later used for sections).

In the "State of the host at fixation" column, spanning vertically: maggots in rest period for about 24 h

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
3		98 h 20 m	1 *larva* in fatty tissue in centre of maggot in segments IX and X. Larva of the slender type. Length: 1.3 mm + 0.2 mm tail. Thickness about 0.25 mm. Along entire length fairly good contact with fatty tissue.
,,		,,	1 *larva* between lobes of fatty body in segment XIII (and perhaps XIV and XV). Larva of the short, compressed type; head, 11 segments, tail. Length: 0.55 mm + 0.15 mm tail. Thickness 0.2 mm.
,,		,,	1 *larva* in fatty tissue in centre of maggot in segment X and XI. Larva of the slender type; head, 11 segments, tail. Length: 1.2 mm + 0.15 mm tail. Fairly good contact with fatty tissue along whole length.
,,	maggots in rest period (7 days old)	,,	1 *larva* between lobes of fatty body against left tracheal main trunk in segments XI and XII. Larva is of slender type, with rather short tail. Unfortunately damaged in preparing. No contact to fatty tissue.
,,		,,	1 *larva* in loose fatty tissue between convolutions of intestine in segments XI and XII. Larva of the slender type; head, 11 segments, tail. Length: 1.15 mm + 0.2 mm tail. Thickness about 0.22 mm.
,,		,,	1 *egg* attached to cuticle, dorsal in segment IX. Proves to be split open chorion. In another place in the same host indications are found of a similar case. Apparently the eggs here have not been normally loosening before the hatching of the larva.
,,		,,	1 *larva* unattached in fatty tissue near centre of maggot, against intestine in segment XII. Larva of slender type; head, 11 segments, tail. Length: 1.05 mm + 0.26 mm tail. Thickness about 0.24 mm.
,,		,,	1 *larva* in fatty tissue in about segment VIII. Larva damaged at posterior end in preparation, but the compress position of undamaged segments shows that it was of the short type, therefore probably just hatched.

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
4		121 h	1 *larva* in fatty tissue close up against tracheal main trunk in segment X. Larva of slender type; probably head, 11 segments, tail, but head capsule and segmentation little marked. Length: 1.2 mm + 0.2 mm tail. Thickness about 0.17 mm. Only at caudal end contact with fatty tissue.
,,		,,	1 *larva* unattached between convolutions of intestine and Malpighian tubes in segment XII. Large specimen of slender type, but segmentation vague and thickness noticeably greater than in former specimens. Length: 1.4 mm + 0.2 mm tail. Thickness 0.26 mm. At both sides laterally an internal organ (gland? trachea?) visible as darker streak, beginning close behind head and running far backwards.
,,	maggots in rest period (8 days old)	,,	1 *larva*. Host with well developed intestinal caeca. Larva lying at the extremity of one of these in segment X with tail attached. Caudal part of larva at right angles to longitudinal axis of maggot, then curved inwards and backwards, so that the head in longitudinal axis is directed freely backwards. Larva of slender type, but segmentation is vague and increased thickness might indicate that it is passing on to the type described beneath. Length: 1.2 mm + 0.15 mm tail. Thickness 0.22 mm.
,,		,,	1 *larva* with tail against tracheal main trunk in segment XIII (?). Contact with trachea is so that when it is torn out of the surrounding tissues the larva remains attached to the trachea. Larva of the slender type; distinct head, 11 segments, tail. Length: 0.8 mm + 0.14 mm tail. Thickness 0.18 mm. *In the same host*: 1 *larva* curled round the same tracheal trunk in segment XI. Head directed forward in fatty tissue. Larva is of the short, compressed type, segmentation indistinct. Length: 0.7 mm + 0.15 mm tail. Thickness 0.14 mm.

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
12		144 h	1 *larva* in or against muscular tissue in anal segment (XIV + XV) and about 1 mm long. Is flat, white, abnormal. Probably remains of dead larva.
,,	maggots in rest for about 3 days	,,	1 *larva* somewhat ventral of the centre of the maggot in longitudinal axis in segments XI and XII. Larva is large, sack-shaped, with loose chitinous cover, segmentation vague, distinct in cuticle only, tail absent. Length: about 2 mm, thickness about 0.4 mm. Lateral canals visible. In the anterior end of the same host lies a chitinous husk, hyaline, perhaps head + segments, exuvium?
,,		,,	1 *larva* ventral median underneath and against fatty body, parallel to longitudinal axis of maggot in segments IX and X, with head directed backwards. Lying with ventral side towards periphery of maggot. Larva large, loose chitinous cover, posterior end pointed but "tail" absent. Length: 2.15 mm, thickness 0.55 mm. The lateral canals are somewhat crinkled, more ventrally a pair of narrower straight canals. Ventral median at regular intervals round groups of tissue (cf. fig. 8).
,,		,,	1 *larva* a little ventral of centre of maggot parallel to longitudinal axis in fatty body, close against convolutions of intestine and Malpighian tubes in segments XI and XII, with head directed backwards. Larva large, sack-shaped with loose chitinous cover with a short tail in which the internal tissues do not penetrate. On head only some loose fatty tissue.
13	maggot in rest for about 4 days	165 h 30 m	1 *larva* in fatty body round tracheal main trunk in segment XII. Larva of the slender type with tail. Distinct head, 11 segments, tail. Length: 0.9 mm + 0.19 mm tail, thickness 0.16 mm. Larva lies in plane at right angles to longitudinal axis of maggot with head directed upwards to close to muscular layer. Tail curled round trachea.

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
13	pupae, fixation on day of pupation	165 h 30 m	1 *larva* in anterior part of pupa in fatty body close to intestinal caecum. Larva is large with loose chitinous cover, tail absent. Length: 2.2 mm, thickness 0.4 mm. Lying with head probably directed forward.
,,		,,	1 *larva* firmly embedded in fatty tissue at posterior end of pupa, near a tracheal main trunk. Larva is small, compressed, but consists of head, 11 segments, tail. Length: (including tail) 0.75 mm, thickness 0.15 mm. In close contact with fatty tissue along entire length.
,,		,,	1 *larva* in pupa, which is dried up through technical error. Larva also dried up, so as to show no details, but has now a length of 2.05 mm and a thickness of about 0.16 mm. Place of the larva and remains of the pupal tissues can no more be analysed.
14	pupae, fixation about 24 h after pupation	191 h 45 m	1 *larva* in posterior part of pupa for $1/4$ to $1/3$ of length of pupa. Larva has (rather vaguely) 13 segments and is entirely milky white. Cuticle lies fairly loose and at the thickest (posterior) end forms a fine spine. Chitinized mouth parts colourless. Length of larva 2.5 mm, greatest thickness 1.0 mm. Larva lying with head directed forward. In the middle of the pupa a circular cavity has been eaten out along $1/3$ of its length so that at the sides there remains only a thin layer of polished, compressed pupal tissue. Organs of the maggot still well distinguishable in the pupa. There seems to be no preference for a special tissue, near head of larva everything has disappeared, at most tracheae pushed aside.
,,		,,	1 *larva* in anterior part of pupa for almost $1/3$ of the pupal length. Damaged in preparing out, must have been entirely comparable to former larva. Cuticle ample, in which, clearly segmented, are the white tissues of the creature. Pupa, in almost entire length eaten away in centre. Remaining layer of tissue has a polished wall and is at the back somewhat thicker than in front.

Set	State of the host at fixation	Time elapsed between pa-rasitization and fixation	Found in preparing:
14	pupa, fixation about 24 h after pupation	191 h 45 m	1 *larva* in centre of pupa along almost entire length. Larva sack-shaped, with loose chitinous cover, in which are compact tissues of which segmentation (13 segments) is only indicated by fine grooves. Internal organs indistinctly visible. Length 4.8 mm, greatest thickness (at $^3/_4$ of body) 1.4 mm. Tail absent. Larva lying with head probably directed forward, is entirely surrounded by loose tissue. No cavity has been eaten out.
15		217 h 15 m	1 *larva* with head forwards. Puparium further almost empty, contains only mouth arma-ture, crop, tracheal trunks, thin chitinous veils (= cover) and remains of intestine (?). Larva is 6.5 mm long, greatest thickness 2.2 mm. Cuticle not loose. Segmentation indistinct, cannot be verified as this specimen is damaged on one side. Lateral canals are crinkled and slightly bulging. The more ventral canals run more straight. Ventral median in each segment a circular complex of white cells. Mouth parts well visible. Tail absent.
,,		,,	1 *larva* with head forwards, firm and compact. Puparium contains of maggot mouth arma-ture, crop, something of imaginal discs, a little intestine, hardly any chitinous veils but sometimes a little muscular tissue and some undeterminable loose tissue. Larva is 6.0 mm long, greatest thickness 2.1 mm. Cuticle not loose, in many parts with hairs and setae. Segmentation is formed more by arrangement of internal organs than by constrictions of the cuticle. Only clear in the middle segments. The lateral canals extend as far as the mouth parts. The more ventral canals are also somewhat crinkled and seem to have contact with each other at the posterior end. The ventral median cell groups are here extended at right angles to the longitudinal axis of the larva. Mouth parts clearly visible. Tail absent.

(Rows 15 and ,, are both marked "pupae, fixation about 2 days after pupation")

Set	State of the host at fixation	Time elapsed between parasitization and fixation	Found in preparing:
16	pupae, fixation about 3 days after pupation	240 h	1 *larva* which entirely fills the puparium. From maggot only remains: cover, chitinous remains of tracheae and a pellet of white tissue = crop. Length: 6.0 mm, thickness 1.8 mm. Internal organs shimmering through as in former specimen, segmentation again indicated by their arrangement. Ventral median fold in cuticle (may be artifact). Mouth parts distinct.
,,		,,	1 *larva* which entirely fills puparium. Length: 6.1 mm, thickness 2.1 mm. Internal organs as above, distinct. Segmentation in cuticle again not pronounced. At posterior end chitinous spike. Mouth parts. Pupa completely eaten.
17	pupae, fixation about 4 days after pupation	259 h 15 m	1 *larva* filling puparium, length 6.5 mm, greatest thickness 2.4 mm. As former larva, but cuticle looser and showing distinct segmentation. 14 segments (somewhat improbable, constriction between last two segments probably artifact). Chitinous spike, mouth parts, cuticle with hairs and setae. Pupa entirely eaten.
,,		,,	1 *larva* filling puparium, length 6.4 mm, greatest thickness 2.2 mm. Like former larva. Cuticle with hairs and setae, but less loose, has a spike. Mouth parts. Pupa entirely eaten.

3. Entries of my own research with microscopic preparations.

Egg in ovary.

Plasm in the whole egg homogeneous (to be seen also in just-laid eggs in toto-preparations in glycerol-gelatin). In transverse sections of ovaries, the egg nucleus was once seen as a vague highly refractive spot. Nucleus with this staining very little conspicuous.

Egg 22 h after deposition.

(Host: feeding maggots). Series 8*a* and 8*b*.

Both in longitudinal and transverse section (cf. photos 1, 2 and 3) the plasm in this stage lies chiefly in a layer up against the chorion; the layer is about twice as thick at the egg poles as in other parts. Inwards, this layer is sharply defined against the centre, which is almost empty and contains only disconnected remains of plasm. The accumulation of apparently empty cleavage

nuclei gives the outside layer of plasm bordering on the chorion a net-like structure. Close under the chorion especially, the nucleoli of the cleavage nuclei are often visible as small, strongly coloured granules. The number of these cleavage nuclei decreases rapidly towards the inside, so that the net-structure stops and the peripheral layer of plasm acquires a more and more homogeneous character on the side towards the centre. The eggs lie practically always close to, or in, the muscles of the host.

Egg 45 h 35 m after deposition.
(Host: feeding maggots). Series 9a and 9b.

. The superficial cleavage has taken place; the blastoderm is formed. It is thicker on one side than on the other along the longitudinal axis of the egg (cf. photo 4).

(At both poles of the egg the blastoderm does not join the chorion, while this protrudes further than the central mass of yolk. In transverse sections the following is found: The chorion is seen as an empty ring for 30μ; then the blastoderm appears first as a central mass of tissue and then as (empty) ring of tissue within the chorion; after 15μ follows the central mass of yolk, which increases in diameter, till after about 15μ, thus 60μ from the pole of the egg, chorion, blastoderm and yolk are closely united. Very likely this condition is an artifact caused by shrinkage in fixation. The regularity with which these layers occur makes it desirable, however, to note their position.)

Occasionally an indistinct longitudinal constriction of the yolk was observed, while in one or two sections sometimes two slightly more refracting ovals in the centre of the yolk could be seen, around which particles of staining matter were arranged radially.

Egg 70 h after deposition.
(Host: maggots in period of rest). Series 10a to d.

In the egg the embryo is visible. In longitudinal section (cf. photo 5) the segmentation is especially visible in the cuticle against the chorion; further through the regular succession of muscular segments. Internal organs, such as intestine, silk glands, etc., visible in longitudinal section, but can be followed better in transverse section, Fig. 2, a to i, viz. a mandibles; b transition stomodaeum to mesenteron with chitinous formation in proventriculus; c thin-walled rostral part of mid-intestine, abnormal and thick anterior division of silk glands; d mid-intestine now thick-walled, contains enclosed yolk elements; e silk glands now more lateral, commencement of Malpighian tubes; f mid-intestine closed; g proctodaeum; etc. In e to h a thinner spot is observable in the chorion.

Cell structure of mid-intestine, silk glands (except anterior part) and Malpighian tubes all very similar: fairly large light nucleus with distinct nucleolus. The intestinal epithelium differs somewhat as the cells are sometimes more decidedly rectangular, while in the wider middle part of the mid-intestine it is single layered with the nuclei lying at two levels.

Larva hatched recently.
(90 h 45 m after deposition of egg). (Host: maggots in period of rest). Series 11b and 11c.

In longitudinal sections the larva, which is slightly curved, is never struck

in its entire length. In photo 6 for instance, the posterior segments are absent. Transverse sections show that the stage of development now reached by the internal organs is usually intermediate between that of the former and that of the following series (10*a* and 12*c*), e.g., as regards the diameter of the mid-intestine.

Anterior portion of larva: First a lot of nerve and wall tissue. Nerve tissue both dorsal and ventral, connection indistinct. Ventral nerve cord, which must begin here, is thin and parts are missing; beside the mid-intestine it is well developed, wider and often shows the ganglia. Only remains of mandibles.

The anterior portion is surrounded by thicker chitin of head capsule. Beginning of intestine has a fairly thick, distinctly chitinous intima. After that, wall thinner and further intestine compressed dorso-ventrally and wall thick with small scattered nuclei (typical stomodaeum structure). In this part the silk glands, lying ventral to the intestine are much wider than the latter. They remain almost cylindrical to the end but decrease in diameter posteriorly and lie more dorsal. They continue far to the back. Along a great part of the length ("middle part") they have a distinct, but small lumen and thick wall.

In the mid-intestine, in the anterior 1/4 or 1/5 part, where the silk glands still lie ventrally, the section is circular and almost entirely filled by the intestinal content (as in 10*a*), after that size increases and intestine is laterally somewhat compressed (as in 12*c*). In this part intestinal content does not entirely fill up the intestine and the peritrophic membrane is more distinct. The Malpighian tubes appear longer in proportion than in 10*a*, they extend further forward. (The place where they should debouch is badly preserved so that there are no particulars about it). Immediately after end of silk glands dorsal to the Malpighian tubes there is a rudiment of gonads on both sides. Gonad tissue damaged and cannot be followed. It almost certainly begins on both sides in the same vertical plane.

Larva fully 2 days old.

(144 h after deposition of egg). (Host: maggot in period of rest). Series 12*c*.

Anterior part of larva damaged and not distinct. Only remains of mandibles. 25μ from beginning of larva can be seen in transverse section: dorsal (brain) and ventral nerve tissue, dorso-ventrally compressed typical stomodaeum. Efferent duct of left silk gland struck, glandular tissue of right. About 40μ further back the para-oesophageal connectives would lie. (Altogether a great resemblance to what I found in the anterior part of the larva in series 13*c*: cf. photo 7).

At this stage there is a distinction between glandular cells of silk glands and Malpighian tubes along the whole length of the glands: the cells of the silk glands are more wedge-shaped, there seems to be an increase in superficial size at the side of the cell opposite to the lumen; the cells of the Malpighian tubes are more flattened and extended tangentially. In anterior part of larva the cells of the silk glands are larger, but in the posterior part of the larva those of the Malpighian tubes are the larger. Silk gland cells are mostly stained darker, nucleolus (especially in front) larger. In both mitosis is often observed.

Diameter of mid-intestine greatly increased, compressed laterally, epithe-lium cells extended tangentially, cf. fig. 3a. Intestinal contents enveloped in distinct peritrophic membrane. Very instructive: transition mesenteron-proctodaeum, cf. fig. 3a to i. Mid-intestine closed caudally. 8μ further the beginning of the proctodaeum can be seen dorsal to it. Malpighian tubes begin gradually to debouch into the mid-intestine. 40μ further transition from mid-intestine to proctodaeum. In the same vertical plane as beginning of opening of Malpighian tubes lie rudiments of gonads, not quite symme-trical, right portion begins somewhat more rostrad, is about 30μ long. (Left portion is torn, length unknown, but certainly begins more backwards than right one). Within proctodaeum chitinous intima visible. Ventral nerve cord well developed. Cuticle double layered, within the outside thin chitinous layer lies a still thinner one, especially noticeable in the middle part of the larva. Dorsal vessel cannot be followed over the entire length of the larva, but is especially well developed above the last part of the mid-intestine (a to c).

Larva fully 3 days old.

(165 h 30 m after deposition of egg). (Host: just pupated). Series 13c.

At the anterior point of larva cuticle folded inwards ventral medial. In this fold lies beginning of stomodaeum. Of mandibles (as in 11c and 12c) connection less clear, as these hard obstacles are broken by microtome. Only scraps remain. About 70μ behind the beginning of larva can be seen in transverse section (cf. photo 7): dorsal brain, ventral nerve cord (contact between these is most clear 20μ further), dorso-ventrally flattened stomo-daeum with thick wall, containing scattered nuclei. Glandular tissue of the right silk gland is struck here and of the left gland the efferent duct (Same as in series 12c). The course of the silk glands proves to be as follows: in the ventral medial fold of the cuticle a common efferent duct for both silk glands opens ventral to the intestine and independently of it. This duct, which is about 32μ long and has a fairly thick wall, is cylindrical in its anterior portion, but for the last 8μ it is oblong in section viz. extended horizontally. After the devision into right and left lateral duct, the glandular tissue begins in the former after about 5μ. The left gland has its own duct of about 40μ length. The silk glands extend far back into the body. The left stops at the opening of the Malpighian tubes into the intestine, while the right runs on for about another 28μ. This makes the position of the rudiments of the left and right gonad, both of which lie just behind the termination of the silk gland, asymmetrical. The rudiment of the left gonad is about 28μ long and stops in the same vertical plane as the right silk gland. The rudiment of the right gonad lies immediately behind it and is about 20μ in length. About 160μ from the beginning of the larva the mid-intestine has begun cylindrically. Its diameter increases so much that it occupies almost the entire transverse section of the larva and the other organs only lie round it in a narrow ring. Mid-intestine is caudally closed before the opening of the Malpighian tubes (at this height there is a dorsal constriction of the hypodermis. This may be an artifact, however). After this the intestine has again an open lumen. The Malpighian tubes debouch into this part of the intestine very gradually (the tubes approach at a slight

angle) which occupies 25–30μ. No difference can be seen between the cells of the intestine and those of the Malpighian tubes. Transition mesenteron-proctodaeum less well preserved; the proctodaeum extends rostrad less far above the mesenteron. Mid-intestine debouches ventrally into proctodaeum. Proctodaeum thick walled, with tall, narrow cells, in which are small scattered nuclei. Proctodaeum to be seen in 17 sections (of 4μ); thickness of walls remains constant, lumen gradually more compressed. There is a hiatus between the first and last group of sections so that probably about 12 sections have been lost. The total length of the proctodaeum would then be about (17 + 12) × 4μ = about 120μ.

Along entire length of mid-intestine the dorsal vessel can sometimes be seen with a distinct (sometimes double layered?) wall, close under the hypodermis. Sometimes it contains large cells with strongly stained nucleoli (leucocytes?).

Cuticle consisting of two layers.

Larva fully 4 days old.

(191 h 45 m after deposition of egg). (Host 1 day pupated). Series 14a and 14b.

The anterior part of larva is struck longitudinally in series 14b (cf. photo 8). At the beginning of the stomodaeum one of the mandibles can be seen. Radial muscles cling to the wall of the stomodaeum. The crop seems to appear at this stage, as a bulging of the stomodaeum. It is surrounded by delicate muscles, opens into the most posterior part of the stomodaeum and extends with the blind end in a rostral direction. At the transition from stomodaeum to mid-intestine, the thin intestinal wall is probably invaginated into the mid-intestine as a narrow canal, after which it bends back again to the beginning of the mid-intestine. A valvula cardiaca seems to be formed. The anterior, widening part of the mid-intestine has a wall consisting of tall, cylindrical epithelium. The mid-intestine widens further considerably. The cells of the epithelium are then flatter.

At this age a differentiation of the wall of the larva into several layers can be seen (cf. figure 4, a to f, tangential sections at various depths). Beneath the thin, structureless cuticle lies a single layered hypodermis with small, but very conspicuous round nuclei (a). Beneath this a compact layer of muscle (b). The layer below this consists of large polygonal cells containing a large nucleus with compact mass of chromatin (c). The cells lie in rigid connection and are only exceptionally separated by narrow intercellulars. This layer is in certain regions followed by a layer consisting of even larger cells with very indistinct boundaries and with large nuclei, which are shown by scattered chromatin (e). In that case, between the two layers there is a very thin inter-layer which contains a few muscular elements and further resembles connective tissue (d). Finally the intestinal contents is found, surrounded by a peritrophic membrane which exactly follows the surface of the epithelium bordering the intestinal lumen (f). In the mid-intestine, however, usually regions are found where the layer mentioned above characterized by its rigid cell connection and the regular arrangement of the cells (c), borders directly on the intestinal lumen.

In the intestine for the first time haemolymph as well as digested fatty tissue is found, lying collected along one side of the intestinal wall. Moreover

there are remarkably large globules, sometimes having circular vacuoles (accumulation of secretions by the intestinal epithelium?). In the last part of the mid-intestine, viz. with its end 500μ from the termination of the intestine a rod of intestinal content lies, more grey in colour and of firmer consistency than the surrounding. It is 400μ long: beginning of formation of the meconium.

The mid-intestine after being closed, continues more narrow. In this part convolutions occur so that it is struck at varying angles. The opening of the Malpighian tubes, which should take place here, was therefore not observed.

After this the mid-intestine opens into the proctodaeum, which, beginning 40μ more rostral lies dorsal to it. Proctodaeum can be seen in about 40 transverse sections, would therefore be about 200μ long. In series 14a the free opening of the proctodaeum is struck longitudinally (cf. photo 9).

The diameter of the mid-intestine at this age and in larvae in older maggots has increased to such an extent that all organs outside it are compressed, and can be hardly recognised, or not at all. Dorsal vessel, therefore, not observed; the ventral nerve cord only below the last narrower part of the mid-intestine; unknown how far Malpighian tubes extend forwards. Even beside the last part of the mid-intestine the difference between silk glands and Malpighian tubes is difficult to see. Cells have the same colour in both, possibly cells of Malpighian tubes more often flattened, but in both the majority are wedge-shaped. On left, at any rate, silk gland ends directly before beginning of rudiment of gonad.

Rudiment of right gonad begins beside transition from mesenteron to proctodaeum and is $14 \times 5 = 70μ$ long. Left lies further back, begins 35μ after end of right and is also about 70μ in length; reaches as far as the external opening of the proctodaeum or beyond it.

(Larva in series 14b lies with head towards posterior end of puparium).

Larva fully 5 days old.

(217 h 15 m after deposition of egg). (Hosts pupated 2 days). Series 15 and 15b.

In series 15 the crop can be seen as a muscular bulging of the stomodaeum. Probably the blind end is directed forwards; this part lies free beside the stomodaeum, but before the debouchment the crop comes to lie *in* a widened part of the stomodaeum. Ventral to the stomodaeum lies the common duct of the silk glands. At the end of the stomodaeum the intestinal wall bears a ring of triangular laminae with a broad base which stick far out into the intestinal lumen and seem to be of a chitinous nature (proventriculus?).

In series 15b the anterior part of the larva is soon filled up with glandular tissue. This makes the other organs lying there indistinct. But there can be observed: well developed, spiky mandibles, the basal parts of which are curved backwards and extend about 30μ further. Above the posterior part of the stomodaeum the brain lies and below it the beginning of the ventral nerve cord can be faintly seen. The para-oesophageal connectives are apparently not struck. Especially on the ventral side of the stomodaeum sometimes radial muscles. Stomodaeum in transverse section principally circular. The silk glands have two dorsal branches arising besides the anterior part of the mesenteron and extending far rostrad, dorsal to stomodaeum.

About 150μ from rostral end of larva, mid-intestine begins, at first small but with directly thick wall, contains remains of fatty tissue immediately. After about 50μ intestine widens and occupies practically the whole surface of the transverse section.

Content of mid-intestine consists at first entirely of remains of fatty tissue; somewhat further back lies a peripheral layer of haemolymph around the central fatty tissue; towards the end this layer becomes wider and finally the intestine contains exclusively haemolymph. In this many vacuoles arise, at first only in the periphery, in the last part of the mid-intestine, so that the content has a frothy appearance.

In series 15b the last part of the mid-intestine is convoluted and opens into the proctodaeum lying dorsal to it. In series 15, however, the last part of the mid-intestine has a slightly different course: the diameter of the middle part decreases gradually; it is then closed after the content has stopped; after the closing the last part continues as a thin straight tube, the wall of which consists of typical mid-intestinal cells with large nucleus; this tube lies against the under side of the proctodaeum, but after the lumina are in contact it stretches out caudally and so reaches its blind ending.

Length of proctodaeum probably about 150μ.

The course of silk glands and Malpighian tubes cannot be followed. Not only are they compressed by the wide mid-intestine, but they are sometimes struck transverse and sometimes sloping as they become more and more convoluted. Moreover there is no distinct difference between the cells of the two organs. The further organs were not found either.

The various layers of cells described in larvae of 4 days old (series 14a and 14b) can be seen well in series 15, but in 15b they are not evident in the anterior part, and are little pronounced in the posterior part.

Larva fully 6 days old.

(240 h after deposition of egg). (Host pupated 3 days). Series 16a.

Apparently the paraffin has not properly penetrated at the embedding. Content of the mid-intestine of the larva broken up in cutting, the splinters have completely spoiled the rest of the larva. This series is useless.

Larva fully 7 days old.

(260 h after deposition of egg). (Host pupated 4 days). Series 17.

The larva for the first 150μ is met as an empty chitinous ring in which sporadic wall tissue lies. After this the cuticle is constricted ventral medial. In this constriction the well developed mandibles stick out. At the height of the mandibles the stomodaeum arises from the constriction and proceeds at first in a dorsal direction. In a more rostral section the blind end of the muscular crop is struck, which, therefore, protrudes forewards. The crop is first free from the intestine, but, (as in series 15) at the opening lies in a widened part of it. The stomodaeum cannot be followed further, as about 20μ further back it is compressed and comes to an end. For some length beside the intestine lies a canal with a wall in which the cell structure is visible, which must be the efferent duct of the silk glands. The course of this, however, cannot be followed in either direction.

About 50µ behind the mandibles in the lower half of the sections the blind end is found of a canal directed backwards and somewhat downwards. It widens out rapidly especially in the horizontal direction and finally reaches both sides, so that the ventral part of the sections is no longer in contact with the rest. The larva, thus, has here a ventral, free fold of skin projecting backwards, which joins on to the mouth of the canal or the cavity. Most probably this is a formation belonging to the mouth parts.

The intestinal canal is rather damaged. It is remarkable that no caudal closing of the mid-intestine is found. Apparently the last part of the mid-intestine has no convolutions and it has an open connection with the proctodaeum which lies in its continuation. The proctodaeum tissue is folded over forwards before forming the part of the proctodaeum which runs backwards. There is, thus, a valvula rectalis. The thinner anterior part of the proctodaeum seems to be contracted at this place where it projects into the wider lumen of the further proctodaeum (cf. photo 10).

The course of the Malpighian tubes and the silk glands can hardly be followed, both on account of want of space and twisting of the organs and of the damage done to it by splinters of the intestinal contents.

In the lumen of the silk glands at this age a mass of secretion is found. The cells of these glands, now apparently active, show vacuoles, arranged along the side of the cell opposite to the lumen. The cell nucleus is little conspicuous as the small amount of chromatin is only scattered about.

4. Discussion of the results of my own experiments.

a. Data concerning the stages of the Parasite in relation to those of the Host.

The results I obtained from the preparing out of the parasite and from the study of the microscopic preparations of the parasite inside the host, differ in several points from those given by the authors I have mentioned above. I will therefore discuss them in detail.

The egg of the parasite for the first 48 hours after deposition, is almost always found attached to the cuticle of the maggot by the tubercle situated at the anterior pole of the egg. In length it is about 0.50 to 0.60 mm. As the muscular layer of the maggot lies close under the cuticle, the egg is practically always in some sort of contact with it. This makes ALTSON's suggestion (1920) that "if, for instance, the egg was lodged among muscles, it would be crushed by the movements of the host larva" improbable, as a position in or amongst the muscles of the host proves to be the normal one. It seems to me rather that, owing to the shape and consistency of the egg, which is smooth and resilient, it is more likely that with any contraction of the surrounding muscles, it will evade injurious effects by following the line of least resistance. In preparing out I often found that eggs would,

so to speak, jump out of a muscle upon which pressure was brought to bear. Maggots in which I found eggs and those in which later on the larvae of the parasite were found, had suffered paralysis at the parasitization followed by violent contractions. It is possible that the angle at which the egg was attached to the cuticle might be changed by the waves of contraction passing through the maggot, but I think it highly improbable that any injury would be done to the egg except in very rare cases. Photo 3 shows an egg that lay entirely surrounded by muscular tissue, about 24 hours after depositions. Here there are no signs of injury at all, the preliminaries to the definite cleavage are in the same stage as in eggs which were not embedded in muscular tissue.

An entirely different point, which can be approached now that the position of the egg within the host is known, is the way in which the deposition of the egg probably takes place. It was only later that I learned that the head of the embryo which develops in the egg is directed towards the tubercle of the egg and that in the ovary of the mother insect it must lie with this pole directed away from the oviduct (WEBER, 1933, p. 539). In preparing out the sexual organs of a laying ♀, I had earlier been able to demonstrate directly that this was in fact their position. Not only did the eggs in the ovaries lie with the tubercle in the direction of the top of the ovarioles, but it could be seen that they kept the same direction as they passed along the oviduct on the way to the ovipositor (cf. Fig. 5).

At the deposition of the egg, therefore, it must be attached by the end that is last to leave the ovipositor. It is higly probable that the attachment will take place at the moment when the point of the ovipositor passes through the cuticle of the host in withdrawal.

Several times I was able to observe the effect of the sting of the parasite upon the cuticle of the host. It is shown in photo 12 in which the inside of the maggot is seen on the left and on the right empty space outside it. In the cuticle of the maggot a passage-like opening remains which is closed on the outside by a thin chitinous layer. This seems to be of a different composition or structure to the normal cuticle, as it absorbs the staining matter much more strongly. The chitin surrounding the sting canal, in a conical field lying with the apex inwards, has also undergone a change and assumed an abnormal colour. The canal caused by the sting is not closed on the inside.

In eggs that have been fixed about 24 hours after deposition

a considerable difference in the distribution of the plasm can be seen as compared to newly deposited eggs. (cf. photos 1, 2 and 3). Although the definite cleavage has not yet begun the initiating for it has progressed considerably. The numerous cleavage nuclei have already migrated to the periphery and lie to a large extent close under the chorion, that is, in the cortical layer. In this movement, as they must have been surrounded by a layer of oöplasm, they have removed this entirely from the centre, and it now lies in a thick peripheral zone. The centre, which has a distincly looser structure, contains only the remains of the deutoplasm.

It is probable that the definite cleavage resulting in the formation of the blastoderm will follow rapidly, and in all the eggs that were fixed a day later, that is, about 2 days after deposition, it has already taken place. There was no further differentiation in the contents of the egg, except a tendency to form "Keimanlage" and "Hüllenanlage" (WEBER, 1933), that is, a ventral and dorsal side, as the cells of the blastoderm in the longitudinal direction of the egg are often higher on one side than the other (cf. photo 4).

After this stage the formation of the embryo and its first development seems to proceed very rapidly, for in an egg fixed 3 days after the deposition, the embryo of the parasite is already completely segmented and all the internal organs are formed. The length of the egg is somewhat increased, to a maximum of 0.75 mm. In most cases it is completely filled, but it sometimes occurs that the thorax and abdomen of the embryo are of a smaller diameter than the chorion. The cause of this is not so easily determined, for it must not be forgotten that the fixative may have had a shrinking effect. However, the difference between thicker and thinner embryos might be an indication that in the latter the peristaltic motion of the intestine has begun and that they have swallowed the liquid contained in the egg. Such specimens would then be very near to hatching.

It is remarkable that these eggs of 3 days old, are hardly ever attached to the cuticle of the host any more. It is only very occasionally that they are still fast, and it that case it is always with eggs where the embryo is less distinctly visible, i.e. not so far developed. The normally developed eggs are always found unattached in the deeper lying muscular tissue of the host and even further inside in the fatty tissue. Apparently the egg

leaves go of the cuticle of the host shortly before the embryo comes out and is passively transported to the deeper tissues by movements of the host.

The segmentation of the embryo is clearly visible in the cuticle lying close against the chorion and is further indicated by a regular arrangement of the muscular segments (cf. photo 5). The embryo at this age proves to be particularly suitable for a study of the internal organs, as the original shape and position of them are very distinct (Fig. 2, a to i). The orientation of the sections as regards dorsal and ventral side was decided upon in analogy to sections through older larvae, where it was shown clearly by the course of the elements of the nervous system.

The alimentary canal runs straight and can be divided into stomodaeum, mesenteron, proctodaeum. The stomodaeum has as yet no definitely cellular wall and is marked exclusively by the chitinous intima. At the beginning lie two strong, hooked mandibles and the end is indicated by a rasplike organ formed by about eight rows of small closely packed chitinous tubercles standing at right angles to the longitude of the intestine. As such chitinous formations in Insects are only found in the proventriculus, I take this to be the end of the stomodaeum. In this region lie a few groups of radial muscles. In the first part of the mid-intestine the wall differs but little from that of the stomodaeum but for most of its length it has a well developed intestinal epithelium, consisting of fairly tall, rectangular cells and often the nuclei seem to lie at two levels which is attributable to mitoses. Except at the beginning and end the mid-intestine contains a mass of yolk which it must have enclosed and, or, liquid taken up out of the egg. The mesenteron is closed caudally and then merges into the proctodaeum, which in the embryo follows in a straight line. The transition is histologically rather vague, so that its peculiar qualities can be better discussed in a later stage. The length of the stomodaeum is not accurately known as it was never struck precisely transverse in the sections, but it will be about 60μ; the mesenteron is about 260μ and the proctodaeum about 35μ.

The embryo has two silk glands, but the first part probably with the efferent duct is not yet well developed. They lie like two cylindrical tubes first ventral to the intestine and afterwards curve gradually upwards, so that for the greater part they are lateral and terminate high lateral. Where the mid-intestine be-

gins they have a diameter of about 25μ, but this soon decreases to 12μ. The anterior thick part has an abnormally distended wall. The wall consists further of a single-layered epithelium, the cell boundaries of which are indistinct. Measured from the beginning of the mid-intestine the length of the silk glands is about 220μ.

There are two Malpighian tubes which run forwards and lie ventral to the intestine. They extend to just beyond the end of the silk glands and are about 40μ long.

The cells of the mid-intestine, the undistended part of the silk glands and the Malpighian tubes, greatly resemble each other in the embryo. They have a fairly large, light nucleus with distinct nucleolus. Only in the intestine the cells seem to be somewhat more distinctly rectangular. The distinction between silk glands and Malpighian tubes consists chiefly in this, that in the former the number of cells surrounding the lumen of the gland is sometimes greater. At this stage the difference is very slight, as has been noted in the youthful stages of other Hymenoptera (DOCTERS VAN LEEUWEN, 1907).

At the posterior pole of the egg, in which the caudal appendage of the embryo lies, the tissue seems to be hung up on two ampullaceous protuberances of the chorion.

Nerve and sexual tissues have not been observed in the embryo, while indications of the dorsal vessel only occur sporadically.

Apparently the larva hatches shortly after the stage described above, as in the maggots that were fixed about four days after parasitization the parasite was never found other than as free larva. I have not been able to gain any certainty about the mechanism of coming out, as an empty chorion was very seldom found. In the egg, from which the transverse sections in Figure 2 are taken, the chorion showed a thinner portion which continued for some length as a groove and seems like a preformed rupture. On the other hand, its position i.e. at the posterior pole of the egg, makes this improbable. In one case empty chorions were found in a host in which several eggs had been deposited, which apparently had not let go of the cuticle in the normal way before the hatching of the larva. The chorions were torn open at the anterior pole of the egg. Considering the powerful development of the mandibles and seeing that it is characteristic of the Alysiidae that these are moved in an outward direction, it seems to me probable that they play a part in releasing the larva.

The classification of the larvae into different types (compressed, slender etc.) was based more upon their general appearance and shape than upon their dimensions.

The young larva has a conspicuous chitinous head capsule, behind which 11 distinct segments lie and possesses a well developed caudal appendage. Shortly after emergence the larva is short and compressed, 0.5 mm to 0.7 mm in length, besides the caudal appendage of 0.15 mm to 0.2 mm length (Figure 6). The head is distinctly the thickest part of the larva and the transition to the other segments is abrupt. In maggots that were fixed at the same age, however, a different type of larva was also found, which apparently rapidly develops from the first type; presumably it represents a following type as it persists in older maggots (Figure 7)[1]. It also consists of head, 11 segments and caudal appendage but has a more slender appearance, as the length here varies from 0.85 mm to 1.0 mm and in some cases the thickness is even diminished. The length of the caudal appendage remains about the same. The internal organs are in many respects similar to those of the embryo. But the mid-intestine increases greatly in size in the posterior part and is there much higher than it is wide, while in the anterior part it is still cylindrical and strictly comparable to that of the embryo. The stomodaeum after a chitinous beginning is flattened dorso-ventrally and has a thick wall, in which many scattered small nuclei are found. This configuration, characteristic of the second part of the stomodaeum, is also found in later larval instars. Both the silk glands and the Malpighian tubes seem to have grown proportionally longer, but run in the same way as in the embryo. Just behind the end of the silk gland a small amount of sexual tissue is found, which begins at the same height on both sides. The nervous tissue is also further developed although it is difficult to follow, it lies both dorsal and ventral of the stomodaeum and below the mid-intestine the ventral nerve chord is distinct and often shows the ganglia.

The function of the caudal appendage of larvae of Hymenoptera has repeatedly been discussed by various authors and it is almost unanimously regarded as a respiratory organ. ALTSON (1920) was also of this opinion with regard to *Alysia*. THORPE

[1] The loose appearance of the cuticle in the specimen shown may be partly an artifact, but the possibility that the creature is just about to moult may also explain it.

(1932), however, in ingenious experiments in which he used the aggregation of the flagellate *Polytoma* and the luminescence of *Bacillus phosphorescens* as indicators of the absorption of oxygen, proved that in the first larval stage of several Ichneumonids the caudal appendage was of no importance for respiration. The appendage found in *Alysia* is of the same type as that of the species THORPE studied. But the fact that in my case I frequently found that the larva was in intimate contact through its caudal appendage with one of the main trunks of the tracheal system of the host, leads me to surmise that the organ here fulfils a special function. And although THORPE proved conclusively that in an environment where the oxygen tension was constant the organ did not take part in respiration, I still think that in a more complicated milieu such as the body of the host, the possibility of a respiratory function should not be denied.

The larva now increases in length, up to say 1.3 mm but preserves the same type at first. After about 24 hours it gradually begins to assume another shape, which may be taken as a transition to the following stage. The diameter increases especially in the posterior segments. This is caused by the increase of diameter in the mid-intestine and the whole process is obviously due to the distention of that organ and the pressure from within which this causes. The external segmentation of the larva becomes less and less distinct and it acquires a spindle-shaped form, pointed at each extremity. The head capsule is still distinctly seen but is more pointed and is no longer the thickest part of the larva. The transition to the segments lying behind it is very gradual. From the outside two internal lateral stripes can be seen, caused by organs which are pressed from within firmly against the cuticle. Presumably these are the silk glands.

Before the next stage is reached a moulting probably takes place, at least I found in one maggot which had a parasite in that stage, a hyaline chitinous husk which faintly showed the segmentation, and assume that this was an exuvium. Beyond this I never found larval skins, so that the ecdysis could not be traced. But I shall return to this question at the end of the chapter, when discussing the probable number of moults.

The larva of the next stage is found for the first time in maggots that have been fixed about 6 days after parasitization, in which, therefore, the parasite larva is fully two days old. The length has increased to a good 2 mm and the thickness in most cases is

more than 0.5 mm. The larva is now sack-shaped, more or less cylindrical in form (cf. Figure 8). Behind the head region there is a slight constriction and the diameter gradually increases to about the middle and then diminishes towards the posterior extremity. There is no longer any head capsule and the caudal appendage has also disappeared; the cuticle terminates at the back in a short, fine chitinous spike. The segmentations are scarcely visible externally. The internal development is continued in the direction begun in the former stage. The mid-intestine has increased in volume along its entire length now, principally by the increase of the vertical diameter, while the intestinal epithelium consists of flat cells which lie elongated along the lumen of the intestine (Figure 3, *a*). The contents of the mid-intestine are enclosed by a distinct peritrophic membrane the formation of which will be discussed in a following stage.

It happened that in a specimen at this stage the transition from mesenteron to proctodaeum could be seen with exceptional clearness (Figure 3, *a* to *i*). The mid-intestine becomes thinner at the end and is then closed. The intestinal canal then continues and is surrounded by exactly similar cells as in front of the closure, so that I see no reason for not counting this portion as still part of the mid-intestine. 8*u*. beyond the closure, dorsal to the mid-intestine proctodaeum-tissue is found, which presently shows a lumen. There is as yet no contact between the two, so that the proctodaeum seems to have a blind termination, which dorsally protrudes somewhat (in this case 40*u*) in a rostral direction. In the same vertical plane in which the front point of the protrusion of the proctodaeum lies the Malpighian tubes commence to debouch into the mid-intestine ventrally. The tubes approach the intestine at a slight angle so that the debouching is gradual and occupies some space (in this case 25 to 30*u*). The cells of the tubes are of the same character along the whole length of the organ and at the opening are not to be distinguished from those of the mid-intestine. This might be taken as an indication that the Malpighian tubes in *Alysia manducator* are of entodermal origin. After the opening of these organs has taken place the mid-intestine runs close under the proctodaeum and the lumina of both come into contact. The wall of the proctodaeum differs remarkably from that of the mid-intestine and consists of long, columnar cells, in which the nucleus, indicated by the chromatin, is elongated radially.

The transverse sections shown in Figure 3, *h* and *i* seem to indicate that the proctodaeum, after narrowing down terminates in a wider portion which curves back and then opens out.

Where the mid-intestine closes sexual tissue is found lateral to it, which presently also appears on the other side. The two halves of the rudiment of the gonad, thus, do not lie quite symmetrically.

The dorsal vessel is found, at any rate in the middle part of the larva. Sometimes large round cells lie in it which must have been floating in the circulating haemolymph.

The silk glands at this age have not become proportionally longer and terminate rostral of the closing of the mid-intestine. In the anterior part of the larva they are in contact with one another and there have a common efferent duct which runs ventrally to the stomodaeum and opens rather further behind independently of it. It sometimes occurs that the body of one gland extends forwards as far as the common duct, while in the other the tissue of the body stops sooner and the gland has an efferent duct of its own, which leads into the common duct. This may be found in the older larva also (for instance in one of fully 3 days old, cf. photo 7). But there is no regularity in this phenomenon as it may sometimes be observed in the right gland and sometimes in the left.

The length of the Malpighian tubes, however, is increased, so that they extend further rostrad in the larva. A discussion of the differences which appear at this stage between the cells of the silk glands and the Malpighian tubes is given in the entries at page 456.

In the anterior part of the larva the nervous tissue is now well developed in the form that persists in the older larvae (cf. photo 7). Dorsal to the stomodaeum lies a distinct bilateral symmetrical brain, while ventral to it begins the ventral nerve cord, with well developed ganglia. The para-oesophageal connectives are always indistinct, but it is remarkable that in all cases where a trace of them can be observed, they, or at any rate the lateral tissue which seems to form a connection between the dorsal and ventral part of the nervous system, lie 20 to 40µ behind the beginning of the ventral nerve cord.

After another 24 hours, when the larva is therefore fully three days old, it has exactly the same external appearance and the measurements are practically the same. The mid-intestine is now still more distended and has assumed a cylindrical shape. The silk glands apparently have increased in length; one of them

(in this case the right one) runs conspicuously further backwards than the other, and even reaches about 30μ beyond the debouching of the Malpighian tubes, while the end of the left gland lies at the beginning of this debouching. The more marked development of one gland than of the other, is a phenomenon often found in older larvae also. It is, however, not always the same gland, but sometimes the right and sometimes the left. Moreover there is no correlation between the development of the glandular tissue at the anterior and posterior end of the gland. Sometimes the gland of which the body extends to close by the common duct runs furthest backwards, but in other cases it is just the gland with the unilateral duct, which extends further caudally. As the rudiments of the gonads usually lie close behind the termination of the silk gland, the uneven development of these organs leads to an assymmetrical position of the sexual tissue.

At the age of about 4 days (thus about 190h after oviposition) the larva is in a stage of pronounced growth. This occurs, thus, about one day after the pupation of the host. Consequently, in this stage we find larvae of very varying size. According to whether the growth period has just begun or has lasted for some time, the length may vary from 2.5 mm to 4.8 mm. The diameter does not increase so much. The external appearance of the larva changes at the very beginning of this period (Figure 9). Thirteen segments are clearly visible, the shape has become more pear-like, the greatest diameter being at about the ninth segment. The fact that externally visible, chitinous mouth parts appear now shows that undoubtedly a new stage has begun. The larva is milky white and carries a fine chitinous spike at its posterior extremity.

Some changes have also taken place internally. In the region of the mid-intestine especially the tissue which surrounds the intestine is now further differentiated, so that there is a sort of "stratification" (Figure 4, a to f). Beneath the thin cuticle lies the single layered hypodermis and this is followed by a well developed layer of muscles, with radial and transverse elements. The tissue lying beneath this must be considered as intestinal epithelium as along the greater part of the mid-intestine it immediately borders the contents of the intestine. But it is a peculiar fact that at certain spots in the anterior part of the mid-intestine there are two other layers between it and the contents of the intestine. The first is a very thin intermediate

layer, which consists for a part of small muscles but further seems like connective tissue and within this there is a layer of very large cells. The walls of these cells are indistinct and the large nucleus is indicated by scattered chromatin. Everything suggests that in these cells there is a lively metabolism. It is not known whether in the mid-intestine of the larva of *Alysia* the intestinal cells have a secretory function in some parts only, but the succession of layers which I found especially in the anterior portion of the mid-intestine seem to me to indicate that this function is local. It would then be the large cells which poured their secretions into the intestinal lumen, and the secretion would be of the holocrine type, which seems probable from the state of the cells and especially of their nuclei. But there is another function that might be attributed to these cells, namely the formation of the peritrophic membrane. It is generally supposed that this membrane is formed in the first part of the mid-intestine. VANCE (1932) gives a minute description of the larva of the Braconid *Chelonus annulipes* Wesm., which in many respects closely resembles that of *Alysia manducator*, and gives a columnar epithelium with long cells lying at the entrance of the mid-intestine as the place where the peritrophic membrane is secreted. In the larva of *Alysia* there is a similar epithelium (cf. photo 8) and although in the microscopic preparations the peritrophic membrane was never in direct contact with it, yet it is not impossible, in analogy with the case described by VANCE, that the epithelium should take part in the formation. WIGGLESWORTH (1939) distinguishes two types of peritrophic membrane, one is of a laminated construction and is secreted along the whole length of the mid-intestine; and the other consists of a uniform layer and originates in groups of cells in the anterior part of the mid-intestine. In some insects (*Polistes, Bombus, Apis*) there may be a combination of these types. I do not consider it impossible that this is the case in *Alysia* also and that the peritrophic membrane here is partly formed by the large cells. The intimate contact existing between these cells and the membrane seems to indicate it. For although a laminated construction is nowhere apparent, the peritrophic membrane here follows the cell surface exactly, which in WIGGLESWORTH's second type does not normally occur. Figures 4, *e* and *f*, seem to confirm my hypothesis.

The secretion of both enzymes and the peritrophic membrane is at any rate a function of the mid-intestinal epithelium and

therefore the large cells must have originated from the smaller ones of which the surrounding layer with more distinct cellular connection is constructed. It seems peculiar at first that between these two kinds of cells there should be a layer of connective tissue.

At any rate it is certain that where both layers occur together, the outside layer with smaller cells is regenerative tissue; the shape of the nuclei, moreover, shows that these cells have not yet come into function. I assume that the path along which the young cells will pass from the regenerative crypt to their final place in the intestinal epithelium is not necessarily at right angles to the intestine, but may approach it at a slight angle. The young cells would then come into contact with the lumen at a more posterior portion of the mid-intestine. In the case given in the illustration the regenerative tissue would surround the functioning intestinal epithelium as a slightly wider tube for a short distance. This regenerative tissue would have been wedged forward between the intestinal cells and the muscular layer of the larva, so that it is not surprising that connective tissue elements which no doubt are normal on the inner side of the muscular layer and at the periphery of the intestine should be found on the inner side of the regenerative tissue as well.

Besides the "stratification" described above, which to a large extent concerns tissues which lie outside the intestine, the alimentary canal itself has undergone some changes. These may depend to some extent upon the change of diet of the larva, for it is at this stage that in the contents of the intestine for the first time fatty tissue is found as well as haemolymph. A crop appears, as a muscular bulging of the stomodaeum, lying with the blind end forwards and the communication with the stomodaeum takes place through a short, narrow canal (cf. photo 8). This is different from the condition which seems to occur in older larvae, where it is usually observed that the blind end of the crop is entirely free from the stomodaeum, but that at the opening it is completely surrounded by a widened portion of it. At the transition of stomodaeum to mid-intestine a valvula cardiaca seems to be formed.

In the last part of the mid-intestine, between the closure and the transition to the dorsal lying proctodaeum there are a few convolutions.

It is conspicuous in larvae of this age, that the still unsymmetrically situated gonads have not only increased in length

but extend further backwards. In one case the gonad was observed to reach beyond the free opening of the proctodaeum. This suggests that the gonads grow backwards. A difference in growth might exist between the rudiments of testes and of ovaria. The number of data collected were not sufficient, however, to give certainty as regards this distinction.

The larvae that are fully 5 days old, are again more uniform in appearance and size. The puparium of the host is now eaten almost entirely hollow and in many cases the larva has reached its ultimate length of 6 mm. The fact that hairs and setae are found upon the cuticle shows that again a new stage has been reached. The cuticle of the head and the last segment, however, is almost entirely smooth. The mouth parts have also assumed another shape and are only different in degree from those of the larvae older still. The external appearance of the larva which is slightly changed also remains practically the same (Figs. 10 and 11). I conclude, therefore, that at this age the final larval stage has been reached.

The greatly distended mid-intestine occupies the greater part of the larva, so that the organs lying outside it are much compressed and it is almost impossible to follow them accurately. The caudal closure of the mid-intestine is still very obvious. Shortly after reaching this stage in the anterior part of the larva the amount of glandular tissue has very considerably increased. This is due to the fact that the silk glands have acquired a dorsal branch. These branches of the lateral glands extend along the dorsal side of the larva as far as the segment behind the head. They arise in the silk glands beside the most anterior part of the mid-intestine.

Although the outward appearance of the larva at the last stage undergoes hardly any change with increasing age, the internal tissues may nevertheless alter. In a larva of some 7 days old, for instance, a great activity was observed in the silk glands. The phenomenon took place in a space of about 300μ, situated in the middle of the larva; unfortunately the further parts of the silk glands were seriously damaged in cutting, so that it is uncertain whether they were also active. In the lumen of the gland a pale yellow secretion was found, the glandular cells showed vacuoles ranged along the side of the cell turned away from the lumen. The nucleus was not conspicuous, as there was only a small amount of chromatin, which was scattered over the nuclear area.

It is not surprising that the silk glands secrete actively at the moment when the larva is about to prepare its cocoon, but I will say a word about the function of these glands, which according to their place of opening should be considered as labial glands. It has struck me, namely, that when the larva of about 4 days old begins to devour the tissues of the host, the cavity that it eats out has a remarkably smooth surface. This might be caused partly by the larva pressing hard up against the tissue, but the surface is so well "polished" that this does not seem to me to be a sufficient explanation, and I think it probable that the larva has exercised some other influence as well. Digestion might produce this appearance, but in that case enzymes would have to flow back from the mid-intestine, which is not likely as at this age the larva possesses a valvula cardiaca. I suggest that it may be the effect of a possibly slimy saliva. BERLESE (1909, I, p. 737) notes the appearance of unicellular salivary glands in the stomodaeum, but I never found these in the larva of *Alysia*. For this very reason I wish to emphasize the possibility of a salivary secretion by the labial glands. The glands, or a portion of them, would have to secrete saliva while the larva was feeding and at a later period, characterized by a change in the tissue, provide silk secretion. HENNEGUY (1904) describes the silk glands as metamorphosed salivary glands, and instances are known in which glands, after having first had a different function, yielded silk secretion in a later phase (WEBER, 1933, p. 420). It would be interesting to examine whether in the silk glands of hymenopterous larvae a similar process could take place. Considering the enormous development in the very first stages it does not seem to me to be impossible [1]).

The caudal closing of the mid-intestine becomes indistinct in older larvae, or it is not found at all. It seems, therefore, to disappear gradually during the last larval stage. The mid-intestine then passes freely into the proctodaeum. Caudal of this transition the first thin part of the proctodaeum is invag-

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[1]) While this paper was in the press my attention was drawn to an article by PFLUGFELDER. He demonstrates cytologically in the larva of *Pontania salicis* Christ. (Tenthredinidae, Hymenoptera) that various products, playing a part in the ingestion and in the formation of the cocoon respectively, are secreted by the same salivary-silkgland in different stages.

PFLUGFELDER, O., 1934. Bau und Entwicklung der Spinndrüse der Blattwespen. Z. wiss. Zool., **145**, 261–282.

inated into the lumen of the wider continuation, forming a valvula rectalis which may be closed up so that a closure of the intestinal canal is effected all the same (photo 10).

The time passed before the pupa (Fig. 12) is formed may vary very much. Even with larvae that came from eggs laid by the same mother insect in maggots of the same size and were further cultivated under the same conditions, the last larval stage endured sometimes for a few days and sometimes for several months. The larva preserves about the same external appearance as it has when it first attains the last stage. As the length of the stationary state varies, it cannot be known whether larvae which have reached it will again proceed rapidly with their development, the probability can not be derived from the condition of specimens growing under the same circumstances. In parasites that lie a long time in the puparium of the host, I observed, however, that the rudiments of legs and wings were visible from the outside through the cuticle, while the larval organs, on the contrary, have become less clear (cf. Fig. 11). I assume that in this case the ecdysis which preceeds the formation of the pupa will shortly take place.

In these specimens, seen from the outside, the Malpighian tubes seem to be in a state of decline. It may be interesting to follow the fate of the fragments seen here, according to the function that these organs have had in the larva. If the Malpighian tubes have performed a normal excretory function, the destruction will take place without complications, possibly with the help of phagocytes. After voiding of the content the tissue will be resorbed and the function taken over by the organs of the imago. In the larva of *Alysia*, however, the Malpighian tubes open into the intestine behind the caudal closure of the mid-intestine; they are therefore in open communication with the anus, and the substances that would be excreted by them, would be deposited in the body of the host. It seems to me improbable, therefore, that the organs will have had the normal excretory function.

The Malpighian tubes often function as "storage kidneys" in Insects and it is not impossible that they have done so here. But in this case the accumulated excretion would have to come into the intestine of the imago at the histogenesis. The same would apply to a number of other groups of globules, of which I became convinced that they acted as storage kidneys, so I will include them in the discussion.

Even in the larvae of only 2 days age, groups of white globules can be seen, lying at the ventral side of each segment. In the older larvae they increase in number and in the pupa similar globules are found scattered in the abdomen. As before the voiding of the meconium the abdomen is greatly distended, they are visible between the still unjoined chitinous pieces of the exoskeleton.

MARCHAL (1890) quoted observations by FABRE (1856 and 1863) on the appearance of white cells in the larvae of Insects. They lay principally in the fat body and proved to contain uric acid. I am convinced that the globules found in the larva of *Alysia* are homologous to these and thus also function as storage kidneys. In this case they are more solitary, as the fatty tissue is only slightly developed. At the emergence of the imago, a white mass consisting of wormlike particles is released as well as the meconium and soon dries up. ALTSON (1920) decides that these must be the "white particles" and I can confirm the fact that after the voiding the scattered white globules have disappeared from the adbomen. Nothing, however, is known about their transport to the intestine. MARCHAL moreover noted uric acid derivatives in the mid-intestine of Insects which had come there "par un processus inconnu" and would be taken up in the meconium. It did not seem to me that they were absorbed into the meconium in *Alysia*, as the appearance of the globules in the pupa was scarcely changed. I therefore come to the conclusion that the globules acting as storage kidneys and apparently even the remains of the Malpighian tubes, during the process of histogenesis of the pupal tissues must be taken up in the intestine. That the intestine must be of great diameter is shown by the fact that in the pupa the white globules lie close under the cuticle.

b. *Summary of deviations from data given in Literature.*

The results I obtained concerning the development of *Alysia manducator* Panz. in *Calliphora erythrocephala* Meig. prove to deviate in the following points from the statements made by other authors:

α. A great part of the development of the parasite takes place in the maggot of the host.

In all cases observed the egg of the parasite hatches fully 3 days after deposition. ALTSON (1920) assumes that this is an exceptionally short time and would reveal its abnormality by

the contorted and shrivelled appearance sometimes noticed in pupae of the host due to damage done to the muscular tissue by the hatched parasite larva. This criterium seems to me to be inadequate. Almost all the pupae which were produced by parasitized maggots of *Calliphora* in my research, were of completely normal appearance. The few exceptions which occurred, were equally common in unparasitized maggots and here it was due to undernourishment. The abnormal form of puparium seems to me, therefore, to depend upon the condition of nourishment of the maggot.

At the pupation of the host the parasite in most cases has reached a second larval stage. It may be that this would not have been the case if older maggots had been parasitized. On the bait out of doors, however, maggots of the size I used were constantly being parasitized even when larger ones were present. In my experiments, also, they were unhesitatingly accepted by the *Alysia* ♀ ♀ as host. I am therefore convinced that in normal conditions a great part of the development of the parasite proceeds within the maggot of the host.

β. The development of the parasite is not qualitatively effected by the stages of the host.

I cannot confirm the statement of earlier authors that the development of *Alysia manducator* would be very slow at first and would take place chiefly after the pupation of the host. On the contrary, I noticed a rapid development at once, which gradually continued until the final larval stage was reached. It is true that about 1 day after the pupation of the host a period of intensive growth comes on, but the change of rate in the development that it causes is entirely quantitative and not qualitative.

As I have stated above, I was not able to ascertain exactly how many moults took place. It is impossible to discover the cast larval skins, as they are either very thin, or come to lie between compressed, chitinous portions of the host's tracheal system which is pushed aside by the parasite. So the number of larval stages must be estimated by other means. I use as criteria: the presence of the caudal appendage, the particulars of the mouth parts and the appearance of a setae-bearing cuticle. The distinctness or indistinctness of the external segmentation must not be used as a characteristic, as it will probably be distinct at the

31

beginning of each stage and become more faint with growth as the thin cuticle is very pliable. The habitus of the larva, however, was often an indication that the other characteristics should be accurately examined. On the grounds of my observations I make the following arrangement:

Stage	Particulars	Duration	Plate
Egg	Attached to cuticle, after fully 2 days free from it.	fully 3 days	Fig. 2, Photos 1 to 5.
Larval stage I*a*	Larva compressed, having caudal appendage.		Fig. 6.
Larval stage I*b*	Larva slender, having caudal appendage.	2 days	Fig. 7 and Photo 6.
Larval stage II	Larva cylindrical, sack-shaped, no mouth parts externally visible.	2 days	Fig. 8.
Larval stage III	Larva pear-shaped, mouth parts externally visible.	1 day	Fig. 9.
Larval stage IV	Last stage, cuticle with hairs and setae.	varying	Figs. 10 and 11.

The transition from each stage to the next is always gradual. Habitus and length begin to resemble those of the following stage by degrees. The development of the internal organs, with a few exceptions, proceeds still more gradually. The times given for the duration of the different stages were noted in the summer months, they may be somewhat altered by external conditions. The development of the host will also be affected by them, and perhaps to a greater extent, so that I assume that there will be practically no change in the relations of the stages of development of the parasite with regard to those of the host.

5. Duration of development from egg to imago.

It is impossible to state the exact length of time that elapses between the deposition of an egg and the emergence of the imago from the host puparium, as the last larval stage varies very greatly in length. The figures given are very various, but there is a considerable agreement amongst them. GRAHAM SMITH (1919) names 25 to 95 days, ALTSON (1920) 33 days and more, on an average 52, MYERS (1927) 35 to 95, a duration of 41 days being quoted as typical, while in my laboratory work I experienced 33 to 90 days, for the most part varying between

40 and 50 days. MORGAN (1929) alone reports more steady results in his laboratory work, viz. about 35 days in the summer, 45 in the spring and autumn and 65 in the winter. It seems as if the more constant weather conditions in Australia affect the time. In Europe, too, there are phenomena which seem to indicate an effect of the climate upon the duration of the development.

Here it has been observed (GRAHAM SMITH, ALTSON) that the individuals of *Alysia* which come out in the spring are larger in size than those which do not hatch until the autumn. The larger individuals come from larger puparia of the host, which leads ALTSON to form the following hypothesis: The wall of larger puparia is thicker than of smaller ones and would protect the parasite better from variations of temperature. The longer duration of the development of the parasite within the thinner puparium wall might therefore be attributed to a retarding effect of the temperature. GRAHAM SMITH (1919, p. 377) was also of opinion that the process went more rapidly at a higher temperature, but still I am not convinced that the thickness of the wall of the puparium would be of such great importance, especially as the puparia are covered by a layer of earth. Moreover, in my research, I found many exceptions to this rule. It often happened that small individuals hatched first and it was not uncommon for large puparia to remain a long time intact. HOLDAWAY even considers that normally it will be the smaller specimens that come out first (HOLDAWAY and FAIRFIELD SMITH, 1932).

I believe that other factors should be taken into account. As I said on page 475 the duration of the last larval stage may vary greatly even in larvae derived from eggs deposited by the same ♀ and in maggots of almost the same size, cultivated under uniform external conditions. Although my experiments were not suited to minute investigation it seems to me possible that this is a case of hereditary factors and that different races might occur. Myself I have only data from the laboratory, where the extremes of long and short development are connected by medium periods, but facts observed by GRAHAM SMITH in nature, where there was distinctly a "spring batch" and an "autumn batch", suggest to me that in the latter case we may assume a diapause.

The results of my cultures make it evident that the size of the

maggot at the time of parasitization, does not effect the moment at which the imago emerges.

Neither is there a definite difference in the length of development of a ♂ or ♀. It is true that the minimum period of 33 days was for a ♂ and for ♀ ♀ it was 38 days, but the range of time for ♂ ♂ and ♀ ♀ observed in 60 to 70 specimens of each sex shows little difference. This is remarkable, as both GRAHAM SMITH and ALTSON state that the ♂ ♂ come out before the ♀ ♀, and I myself in the spring of 1939 found ♂ ♂ on the bait on April 27th and ♀ ♀ not until May 11th. Moreover I could not observe any constant difference between the ♂ ♂ that proceed from eggs of fertilized and unfertilized ♀ ♀, the duration of the development is sometimes long and sometimes short. To make certain about this it would be necessary to know whether fertilized ♀ ♀ always deposit only fertilized eggs. As there is an organ in the ♀ which owing to its location and form may probably be taken as a spermatheca (cf. Fig. 5) this is not necessarily the case as the ♀ could regulate the supply of sperma at will.

6. Number of descendants of Parasite and Host.

To estimate the value of a parasite for biological control of a host, it is important to compare the number of descendants produced by each under equal conditions. Different methods are used to obtain data concerning this ratio. Several authors for instance count the number of eggs in the ovaries. GRAHAM SMITH (1919) stated that the ovaries of an *Alysia* ♀ contained at least 549 eggs and ALTSON (1920) found in 12 ♀ ♀ a variation from 325 to 416 eggs with a mean of 366. The number of eggs in the ovaries is not necessarily a criterium of the number of eggs that are deposited, neither can the number of adult descendants be immediately deduced from the number of eggs that are deposited. AHMAD (1936) also, pointed out that in his investigations the number of parasites that actually hatched could vary exceedingly, and was of more importance than the number of eggs that a ♀ deposited, while ALTSON ascertained that with *Alysia* in confinement only 9.89 % of the above stated average egg capacity developed into mature wasps. In my research also the *Alysia* ♀ ♀ stung readily, but this only yielded a poor number of imagines.

In this respect SALT's data (1932) are of more value. He determined the actual reproductive rate of *Alysia* as being 101

and the potential reproductive rate of *Lucilia sericata* as about 1000 by observing how many adult ascendants were produced maximally in the laboratory in these species; in nature the number of descendants of both would be less. On the ground of these numbers he came to the conclusion that controlling *Lucilia* by *Alysia* could never lead to successful results.

From various data given in literature I believe the case to be different with *Calliphora erythrocephala*. As regards this species of host GRAHAM SMITH (1916) remarked that the number of descendants from one ♀ per season is usually very much exaggerated. In a large experimental cage out of doors, where there were naturally practically no enemies and there was always a sufficient supply of food, he determined this as 130, while he assumed that in nature it would be much less. This would be largely attributable to the fact that a very great number of young flies die before they are sexually mature and so would never come to depositing eggs. SALT gave 101 as actual reproductive rate of *Alysia* and GRAHAM SMITH quoted a case in which an *Alysia* ♀ deposited 206 eggs from which 193 living, full grown parasites developed. When these numbers of the parasite are compared to those of the host the position of *Alysia* with regard to *Calliphora* proves to be decidedly more favourable than to *Lucilia*, so that under conditions favourable to the parasite the control of *Calliphora erythrocephala* by *Alysia manducator* is not altogether out of the question. But it will first be necessary to study the conditions in nature as regards both parasite and host.

As a rule flies deposit their eggs in such numbers that the source of nourishment is in no way adequate for the developing maggots. HOLDAWAY showed how great the reduction of a maggot population of *Lucilia sericata* became by "overblowing" which SALT had pointed out before. It must still be ascertained to what extent this applies to *Calliphora* as in the mutual competition of maggots there is a chance that the parasitized specimens also succomb. It is quite likely that such a research might reveal facts which explained the remarkable differences of percentage of parasitization in summer and autumn that GRAHAM SMITH found for *Calliphora* as host of *Alysia*. These percentages were for instance as high as 10 and 43 % respectively, although the parasite was abundant during the whole season and was observed to attack maggots from May 30th to November 1st. Moreover the phenomenon, mentioned in the In-

troduction that a parasite might have a preference for the species of host in which it had gone through its own larval development might play a part here.

<div align="center">CHAPTER V</div>

<div align="center">SUPERPARASITISM AND MULTIPARASITISM</div>

The term superparasitism was introduced by FISKE (1910, p. 89) to indicate a case in which "any individual host is attacked by two or more species of primary parasites, or by one species more than once". Later the term was confined by SMITH (1916, p. 486) to "that form of symbiosis occurring when there is a superabundance of parasites of a single species attacking an individual host insect" (cf. SALT, 1934, p. 456). The case in which a host is parasitized by two or more species of parasites was afterwards given the term of multiparasitism (cf. IMMS, 1937, p. 322).

1. Superparasitism.

a. Possibility of superparasitism.

The chance of superparasitism is naturally very much effected by the circumstance of whether the full grown parasite in depositing its eggs can distinguish unparasitized specimens of the host from those that are already parasitized. SALT (1937) described such a case in which *Trichogramma evanescens* Westw. recognised the parasitized eggs of *Sitotroga cerealella* Olivier with olfactory organs in its antennae from an odour left by the previous visitor, while when this odour was artificially removed a second discrimination occurred at the insertion of the ovipositor through a chemical sense localized there, which then prevented the deposit of a second egg in the host. *Collyria calcitrator* Grav. as parasite of *Cephus pygmaeus* L. and *Ibalia leucospoides* Hochenw. as parasite of *Sirex cyaneus* (? *juvencus* L.) SALT stated to be able also to avoid superparasitism.

With *Alysia manducator* there is no such distinction. The parasites discover putrifying meat from a great distance led by an olfactory sense, as shown by LAING's experiments (1937). In discovering the maggots that inhabit it, sensory organs in the ovipositor-sheath would probably take part, which ALTSON (1920) described as olfactory, but which MYERS (1927) considers to be tactile; not only are different species of Insect larvae in

the meat parasitized, but the fact especially that only moving maggots are stung, supports the correctness of MYERS' opinion. In serial sections of the ovipositor itself I did not find any sensory organs and those in the ovipositor-sheath do not appear to enable the parasite to distinguish a parasitized host as such. I repeatedly observed in the laboratory that parasitized maggots were unhesitatingly restung, and I was able to make sure that an egg was then deposited. As I am convinced that there is no difference of behaviour between parasitized and unparasitized maggots (cf. Chapter III, p. 433) it seems to me probable that the distribution of eggs by *Alysia* over the available number of hosts is quite arbitrary.

SALT (1932) pointed out that in cases where the ♀♀ of a parasite frighten each other away and sting slowly and elaborately, a host is secure from super-parasitization during the whole process of oviposition. With *Alysia* this is not the case. A maggot may in fact be attacked by two or more ♀♀ at the same time and moreover the ♀♀ sting for the whole day and under all weather conditions. Moreover when, through the exhaustion of the parasitizing ♀, the paralysis of the host is no longer complete and the still moving maggot can be immediately attacked again, the possibility of super-parasitization seems to be increased. On the other hand, the attacks of an exhausted ♀ are often without result. For the rest, every factor for a random distribution is present. And yet the number of cases of super-parasitism usually will be small, as normally the numbers of the host will be far above those of the parasite.

b. *Mutual competition between parasite larvae.*

It has been established that with various species of parasites in cases of super-parasitism in the host only one specimen reaches the fullgrown stage. There must therefore be a struggle for existence between the developing larvae, in which only one survives. The competition may be of different kinds. RIETRA (1932) stated that in the caterpillar of *Ephestia Kühniella* Zeller the larvae of *Nemeritis canescens* Grav. during the first larval stage passed to the posterior end of the host. There they came into contact with each other and fights took place in which the strongest with well developed mandibles inflicted such wounds upon its rivals that they succombed. SALT also (1932) told that between the larvae of *Collyria calcitrator* in the host *Cephus*

pygmaeus similar active battles took place, which here transpired chiefly in the second larval stage. In most cases, however, the competition will take another form, but lead to the same result, because, as IMMS (1937, p. 325) expresses it "the individual most advanced in its development gains the ascendancy by starving out its confrères, either through devouring the host, or by rendering it unsuitable as a source of food".

With *Alysia* too, when several eggs are deposited in the host, one at most will develop into a full grown parasite, which may be either of the female or the male sex. To trace the nature of the competition that arises I collected data upon the development of parasites in about 60 maggots which had been twice stung in the laboratory. Between the two parasitizations lay an interval of at most 1 hour 45 minutes. It happened sometimes that after an insertion of the ovipositor that I had thought too brief for a successful oviposition an egg was nevertheless deposited so that occasionally 3 eggs were found in one host. Independantly of the number of eggs the development at first proceeds at exactly the same rate as in a case where only one egg is deposited in the host. In super-parasitization thus I find also:

after 1 day: the eggs are attached to the cuticle, actual cleavage has not yet taken place.

after 2 days: eggs attached to the cuticle, blastoderm formed.

· after 3 days: eggs loose in deep-lying tissues, within chorion embryo visible in most cases.

after 4 and 5 days: larvae of the first stage, both of the compressed and of the more slender type.

As soon, however, as one of the larvae has reached the second stage, there is practically nothing more to be found of the other specimen. And yet, as in the maggots of the same set fixed at an earlier stage more than one egg was always found, more than one parasite must have been present. The only indication that even during the first larval stage the strongest individual might have an injurious effect upon the development of its rival, would be that usually a slight difference in the degree of development can be observed between the two. When one specimen has grown into the slender type, the other is almost always still in the compressed stage. But even here there is an occasional exception.

In one case only in a puparium of the host fixed nine days after its parasitization and in which a parasite was found which

had just attained the last larval stage, the second larva, probably already killed, could be observed (cf. Photo 11). It had apparently continued to develop after the first larval stage, as the mid-intestine was distended. The content of the intestine consisted of haemolymph, but was of an abnormal, very fine consistency, a condition that may have arisen after the death of the individual. The most remarkable thing, however, was the great difference in size between the two larvae. Unfortunately it could not be accurately measured in the serial sections in question, but the length was something less than 6 mm and about 0.5 mm respectively. In the smaller larva no damage to the cuticle could be observed.

The data collected upon the development of the parasite larvae in superparasitism are thus not sufficiently extensive to enable me to say with certainty of what nature the struggle is. The first development proceeds in both parasites at about the same rate and the differences occurring between the two individuals are only of degree. It seems to me probable, however, that when the strongest larva has reached the second stage it will have so much power over its rival that it will dispose of it in a short time. The dead specimen seems to shrivel up quickly, or is crushed by the movements of the host, which the thinness and the pliability of the cuticle would make possible, so that as a rule the remains are not to be found. I consider it to be impossible that active battles take place. The sudden end of the strife suggests that the weaker specimen is injured earlier by the presence of its rival. Signs of this, however, would not be possible to trace.

2. Multiparasitism.

Alysia manducator is not the only parasite of *Calliphora* that is attracted by the smell of putrifying meat. LAING (1937) and JACOBI (1938) have also observed this in the Chalcid *Mormoniella vitripennis* Wlk. Where, therefore, different species of parasites of one host meet in the same milieu, there is the possibility of multiparasitism.

According, GRAHAM SMITH (1916 and 1919) has noted that in pupae of *Calliphora* which had been infected by *Melittobia acasta* Wlk., *Dibrachys cavus* (? *Pteromalus cavus* Wlk.) or *Mormoniella vitripennis* Wlk. there were specimens which already contained a larva of *Alysia*. There was no minute observation

of the consequent rivalry, but in every case the larvae of the Chalcids developed at the cost of the Braconid, which was destroyed. The number of imagines of *Alysia* that hatch may, therefore, be limited by secondary infection of pupal parasites[1]). These do not occur everywhere, however, so that the effect is confined within limits. *Melittobia acasta* seems to avoid shady places and *Mormoniella vitripennis* has only a small power of digging and therefore only infects pupae that lie on the surface.

The maggots of *Calliphora* are also attacked by other species of parasites. GRAHAM SMITH notes that besides *Alysia* they are subject to attack by the Braconid *Aphaerata cephalotus* Hal. and by Cynipids of the genus *Figites*. Small maggots especially would here be the victims. No investigation was made, however, of a possible rivalry with *Alysia*.

In my research, from the puparia of *Calliphora* that I had collected outside, one specimen of *Figites* sp. came out and several specimens of an Ichneumonid probably of the genus *Atractodes* also mentioned by GRAHAM SMITH. The latter specimens showed no inclination in the laboratory to parasitize any of the stages of *Calliphora* so that neither here could an eventual rivalry with *Alysia* be studied.

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[1]) A further limitation of the emerging imagines of *Alysia* may be caused by predators of pupae. GRAHAM SMITH (1919) mentions the Coleoptera *Creophilus maxillosus* L., *Necrophorus humator* F., *Hister cadaverrinus* Hoffm. and *Pterostichus madidus* F. as such.

On imagines of *Alysia* I sometimes found Mesostigmata of the genus *Macrocheles*. As these are not known as parasites, it must have been a case of phoresy. My special thanks are due to Dr A. C. OUDEMANS for the trouble he took in identifying these specimens.

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LEGEND

Figure 1: Longitudinal section through maggot of *Calliphora erythrocephala*, diagrammatic, showing position of segments. After Lowne (1890-'92). ph. = pharynx; cr. = crop; ch.s. = chyle stomac; i = haemal curve of the intestine; d.v. = dorsal vessel; i.d. = imaginal discs; pr. = proventriculus; s.g. = salivary gland; r. = rectum.

Figure 2 (*a* to *i*): Transverse sections through egg of *Alysia manducator*, 70 h after deposition.
Organs of embryo, *a:* mandibles, muscles in oesophageal area; *b:* transition stomodaeum — mesenteron with rasp-like organ in proventriculus, radial muscles; *c:* rostral part of mid-intestine, anterior division of silk glands; *d:* mid-intestine with enclosed yolk, silk glands; *e:* mid-intestine, silk glands lateral, Malpighian tubes; *f:* caudal closure of mid-intestine, Malpighian tubes; *g:* proctodaeum; *h:* caudal curve of proctodaeum; *i:* "suspension" of caudal appendage.

Figure 3 (*a* to *i*): Transverse sections through posterior portion of larva of *Alysia manducator*, stage II.
a: mid-intestine, dorsal vessel, silk glands, Malpighian tubes, ventral nerve cord; *b:* mid-intestine, dorsal vessel, Malpighian tubes, ventral nerve cord, fatty tissue; *c:* mid-intestine closed, dorsal vessel, gonad, Malpighian tubes; *d:* mid-intestine, Malpighian tubes, gonads, blind end of proctodaeum (directed rostrad, dorsal to mid-intestine); *e:* proctodaeum, mid-intestine, ventral nerve cord; *f:* as in *e*; *g:* proctodaeum, dorsal caudal appendage; *h:* rectum; *i:* as in *h*.

Figure 4 (*a* to *f*): Longitudinal sections at various depths through larva of *Alysia manducator*, stage III.
a: hypodermis; *b:* as in *a*, layer of muscles; *c:* as in *b*, regenerative cells; *d:* as in *c*, layer of connective tissue; *e:* as in *d*, secretive tissue; *f:* as in *e*, haemolymph in peritrophic membrane. (In *b* to *f* glandular tissue is struck above on the right).

Figure 5: Gonads of *Alysia manducator* ♀, imago, dorsal view, dissected. a. = ovipositor; l. = ovipositor sheath; m. = musculus retractor aculeae; g. = ovaries; o. = oviducts; s. = spermatheca; a.g.= accessory glands; c. = sternite X.

Figure 6: Larva of *Alysia manducator*, stage I*a*, semi-dorsal view, head directed to the right.

Figure 7: Larva of *Alysia manducator*, stage I*b*, lateral view, head directed to the left.

Figure 8: Larva of *Alysia manducator*, stage II, lateral view, head directed to the right, silk gland, Malpighian tube, ventral complexes of excretory globules.

Figure 9: Larva of *Alysia manducator*, stage III, lateral view, head directed to the left, mouth parts.

Figure 10: Larva of *Alysia manducator*, stage IV, lateral view, head directed to the left, branched silk gland, muscular tissue, Malpighian tube, excretory globules; (mouth parts of same, front view, greatly magnified).

Figure 11: Old larva of *Alysia manducator*, ventral view, head directed to the left, rudiments of legs and wings; (mouth parts of same, front view, greatly magnified).

Figure 12: Young pupa of *Alysia manducator* ♂, ventral view, not yet pigmentated.

Figure 13: *Alysia manducator* ♂, imago, dorsal view.

Photo 1: Transverse section through egg of *Alysia manducator* in maggot of *Calliphora erythrocephala*, 22 h after deposition. Cuticle and muscular tissue of host.
250 ×.

Photo 2: Longitudinal section through egg of *Alysia manducator* in maggot of *Calliphora erythrocephala*, 22 h after deposition. Fatty and muscular tissue of host.
130 ×.

Photo 3: Longitudinal section through egg of *Alysia manducator*, embedded in muscle of maggot of *Calliphora erythrocephala*, 22 h after deposition.
130 ×.

Photo 4: Transverse section through egg of *Alysia manducator* in maggot of *Calliphora erythrocephala*, 45 h 35 m after deposition. Muscular tissue of host.
370 ×.

Photo 5: Longitudinal section through egg of *Alysia manducator* in maggot of *Calliphora erythrocephala*, 70 h after deposition. Cuticle and fatty tissue of host.
130 ×.

Photo 6: Longitudinal section through major portion of larva of *Alysia manducator*, stage I, in fatty tissue of maggot of *Calliphora erythrocephala*, 90 h 45 m after oviposition.
130 ×.

Photo 7: Transverse section through anterior part of larva of *Alysia manducator*, stage II (dissected from the host), 165 h 30 m after oviposition. Brain, stomodaeum, ventral nerve cord, ventral right: body of silk gland, ventral left: efferent duct of silk gland.
130 ×.

Photo 8: Longitudinal section through anterior part of larva of *Alysia manducator*, stage III, in pupa of *Calliphora erythrocephala*, 191 h 45 m after oviposition. Mandible, stomodaeum with radial muscles, crop, columnar epithelium, valvula cardiaca, mid-intestine with haemolymph, silk glands.
130 ×.

Photo 9: Longitudinal section through posterior part of same. Mid-intestine, intestinal convolutions, proctodaeum, anus.
130 ×.

Photo 10: Transverse section through valvula rectalis of larva of *Alysia manducator*, stage IV.
250 ×.

Photo 11: Longitudinal section through posterior part of probably already killed larva of *Alysia manducator* in fatty tissue of pupa of superparasitized *Calliphora erythrocephala*.
130 ×.

Photo 12: Longitudinal section through cuticle of maggot of *Calliphora erythrocephala* where it is pierced by ovipositor of *Alysia manducator*. Direction of sting from right to left.
130 ×.

In figures 2 to 4 the numbers between the drawings indicate the distance between the sections reproduced.

Fig. 1

d.v.   i.   ch.s.   cr.   ph.

r.

s.g.   pr.   id.

XV XIV XIII XII XI X IX VIII VII VI V IV

4 mm

a.   30 μ   b.   25 μ

d.   45 μ   145 μ   e.   35 μ   25

g.   25 μ   h.   15 μ   i.   25 μ

Fig. 2

10

5

Fig. 3

Fig. 4

Fig. 5

500 μ   Fig. 6

500 μ   Fig. 7

500 μ   Fig. 8
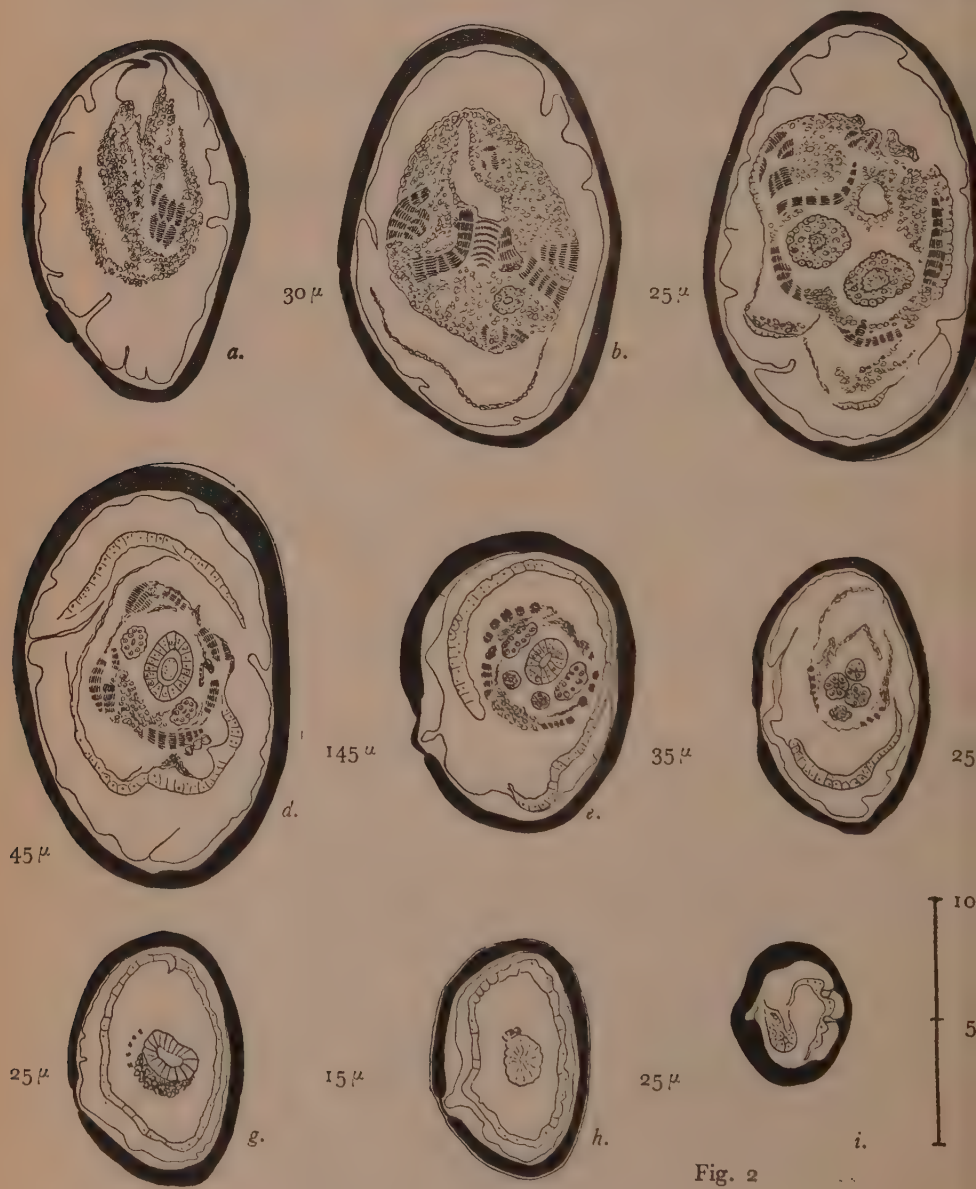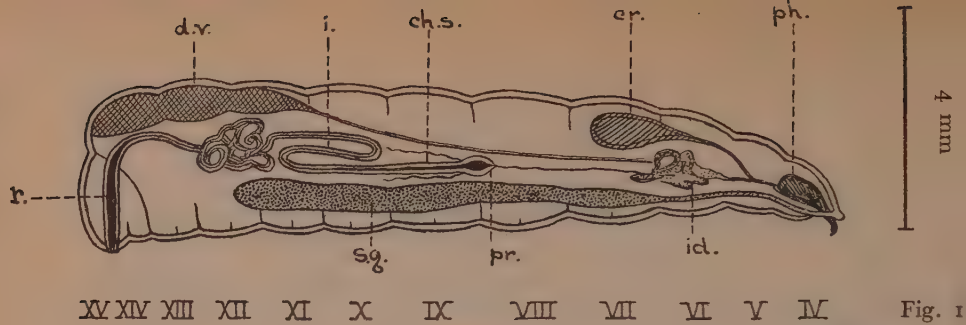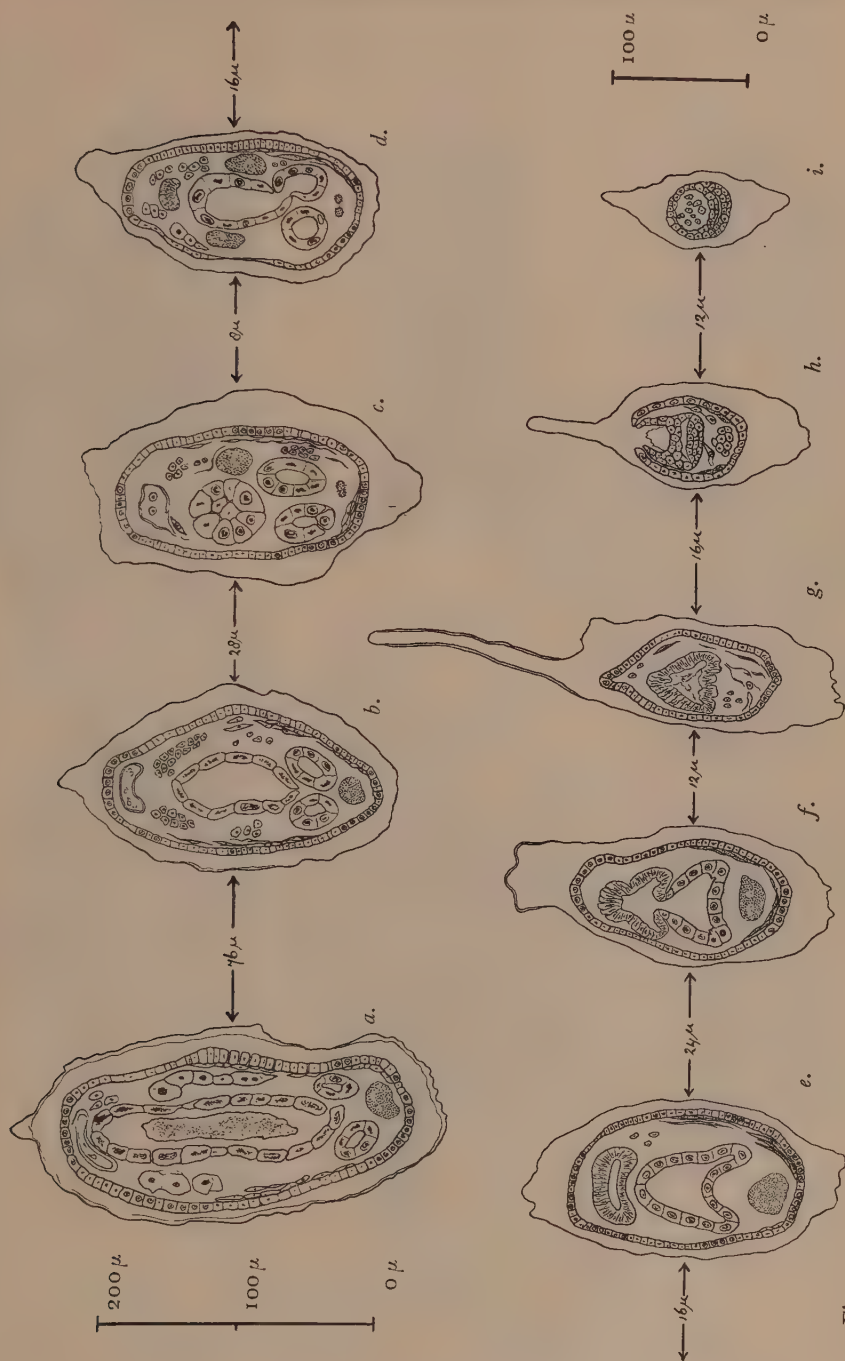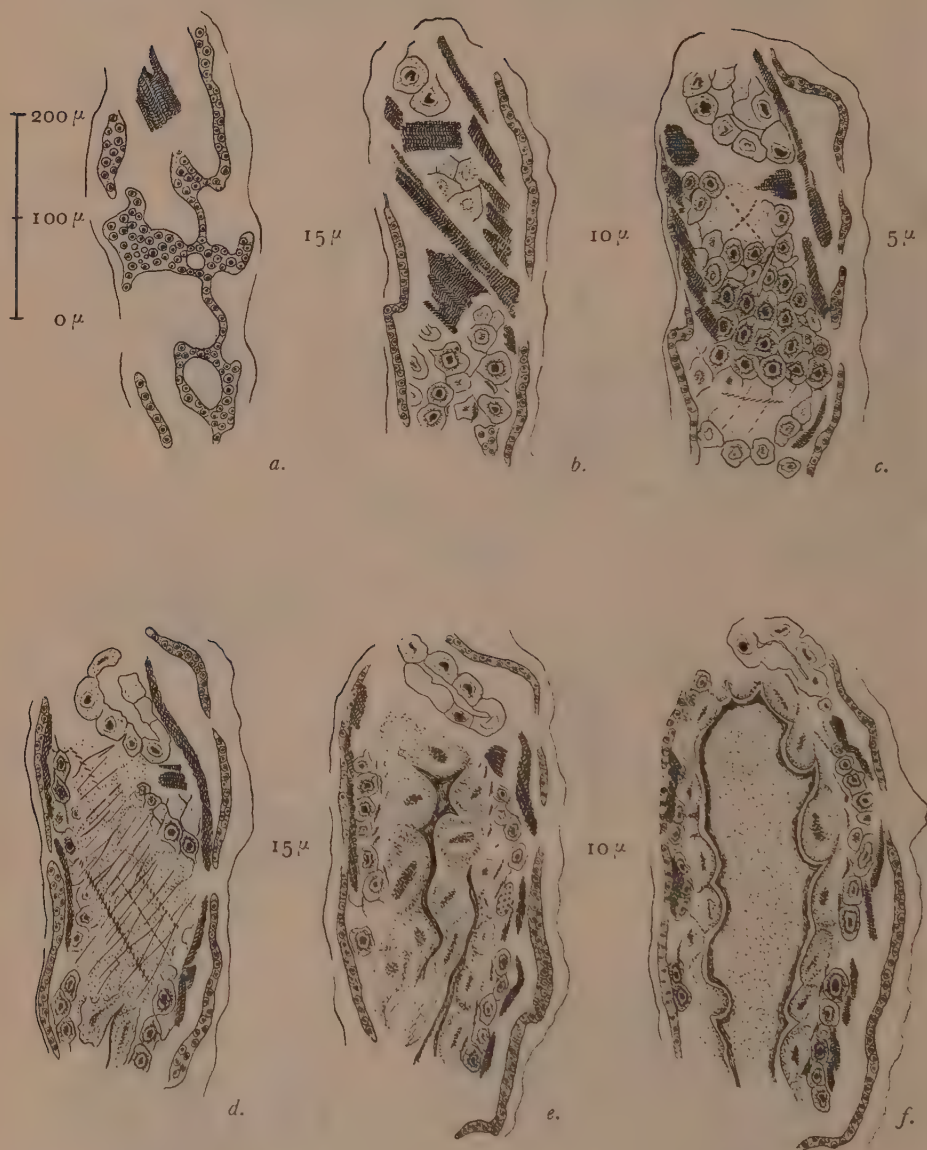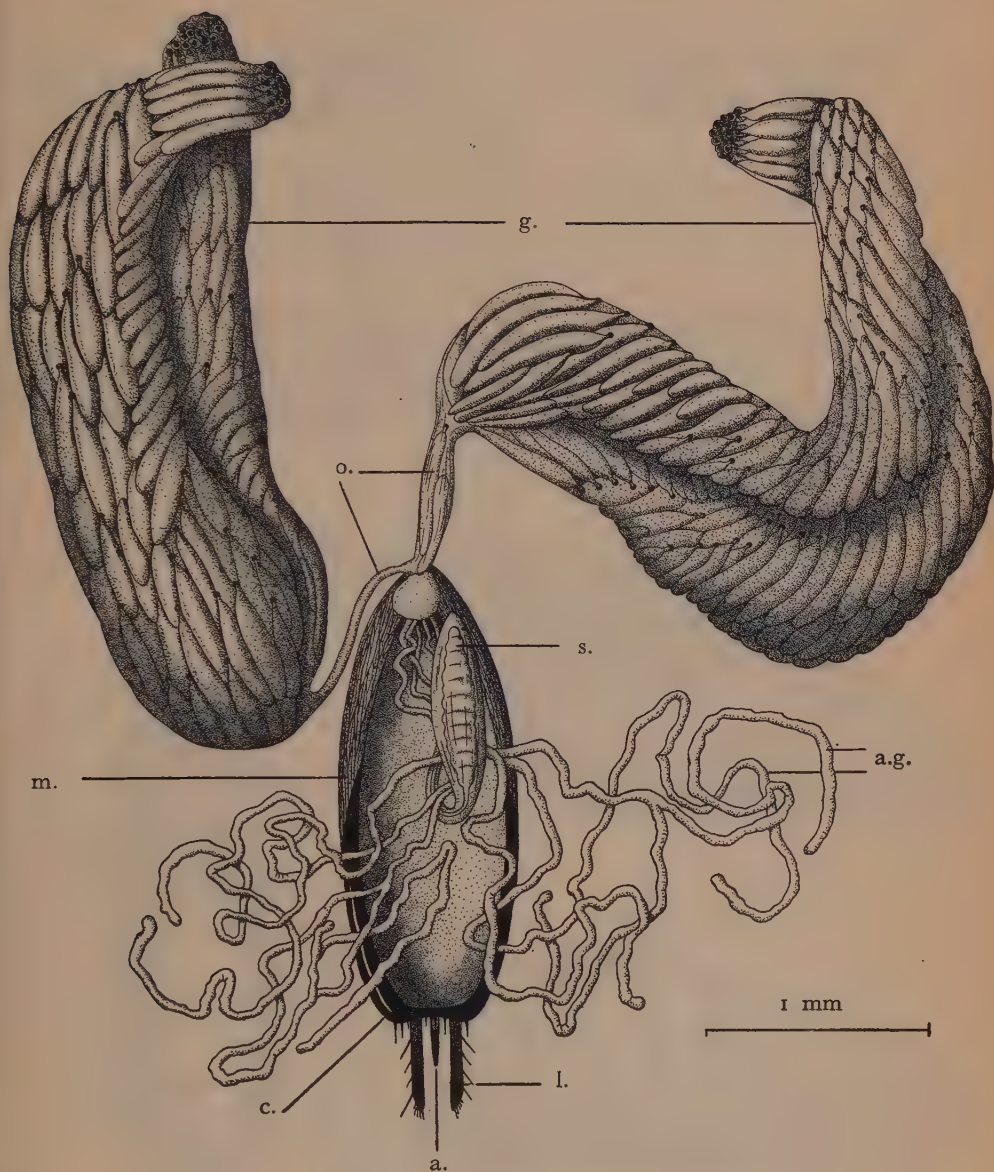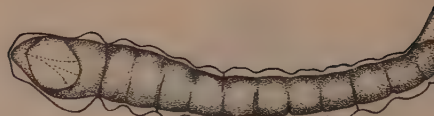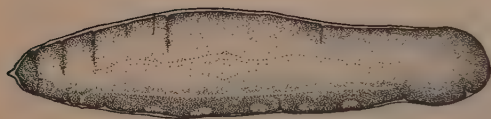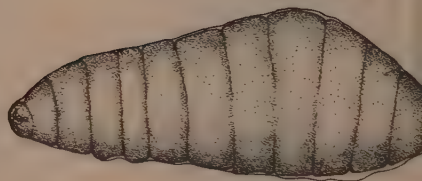
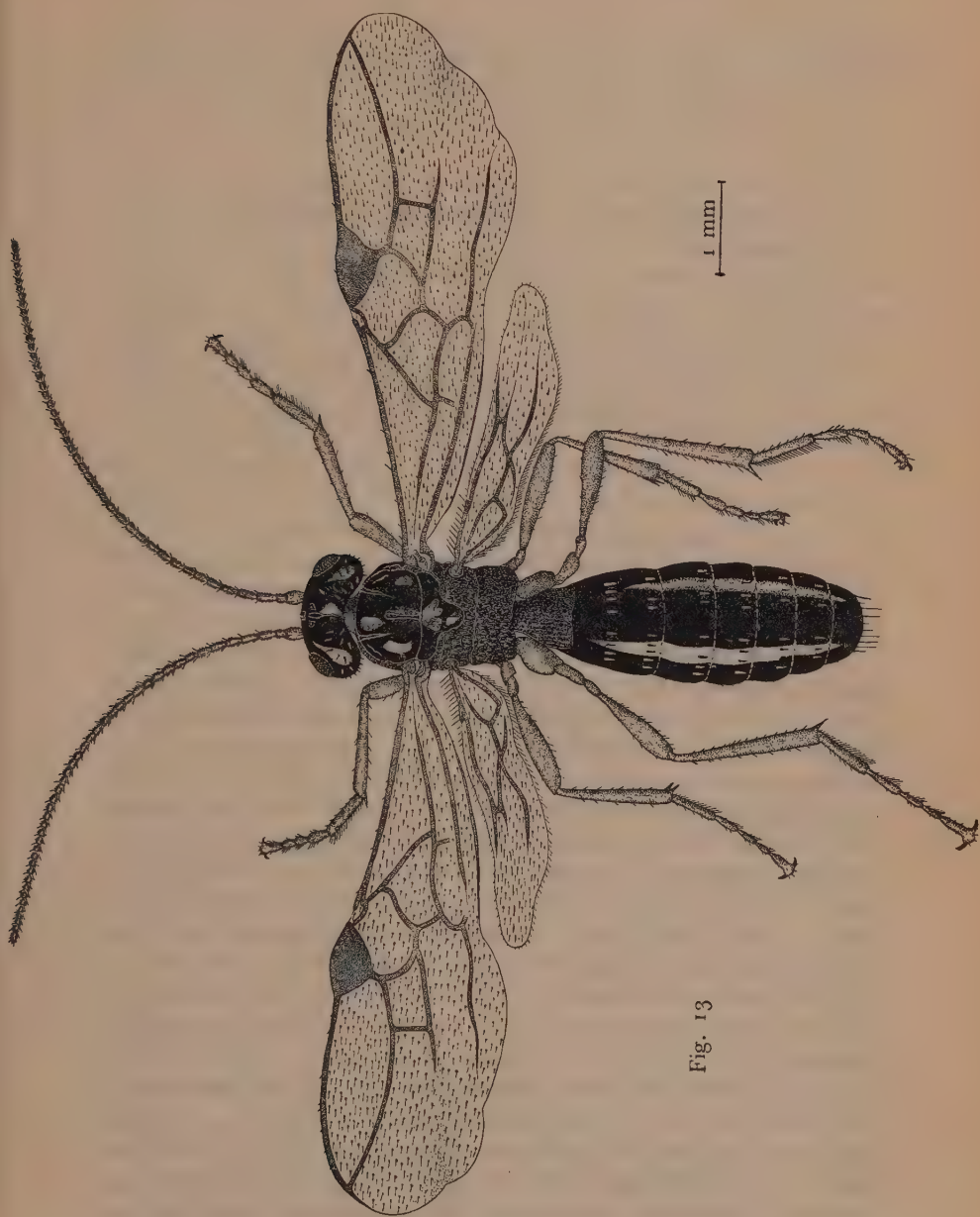500 μ   Fig. 9
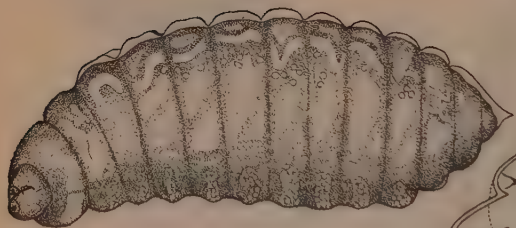
1 mm   Fig. 10

1 mm   Fig. 11

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Fig. 13

1 mm
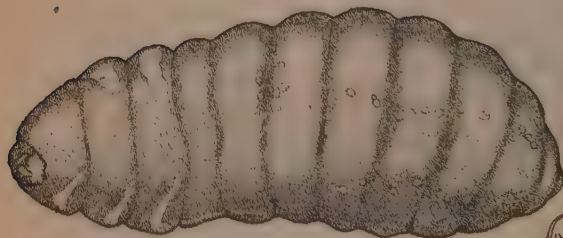
COMPTES RENDUS DE LA SOCIÉTÉ NÉERLANDAISE DE ZOOLOGIE

Séance du 20 janvier 1940 à Amsterdam

N. Tinbergen (Leyde). l'Analyse de l'organisation sociale des animaux.

Voir: the Amer. Midlands Naturalist 21, 210 (1939).

Séance du 24 février 1940 à Amsterdam

1. H. P. Wolvekamp et Mlle M. Vreede (Leyde). l'Oxygénation du sang d'*Arenicola marina L.*

Voir: Arch. Néerl. Physiol. 25, 265 (1940-41)

2. G. C. Hirsch (Utrecht). Unsere neueren Einsichten in die Restitution der spezifischen Produkte im Pankreas und in den Speicheldrüsen bei Säugetieren.

Eigene Untersuchungen und Untersuchungen der Herren Dr. Järvi und Sluiter in meinem Laboratorium haben unsere bisherigen Einsichten in die Frage nach der Restitution von spezifischen Produkten in den genannten Drüsen teils angefüllt teils verändert. Der Aufbau der neuen Stoffe (Enzym-Eiweissträger im Pankreas, Schleim und ein spezifisches Eiweiss in den Speicheldrüsen) geschieht durch die Zusammenarbeit von wenigstens 3 verschiedenen Bestandteilen der Zelle: der Mitochondrien, des Zellplasmas und der Golgi-Körper. Im Anfang der Restitution verändern die Mitochondrien ihre Form; kleine Körnchen begeben sich von ihrer Oberfläche in das Golgi-Feld; deren weiteres Schicksal ist noch zweifelhaft. Durch das Protoplasma wird vom Blut Vitamin C aufgenommen und zum Golgi-Feld transportiert. Die eigentliche Fabrikationsstätte der spezifischen Produkte sind die Golgi-Körper. Sie kommen zuerst in Form einer Präsubstanz vor, welche mit Metallen mikroskopisch gleichmässig durchimprägniert wird; diese Präsubstanzen hängen als Balken meist zusammen. In der Präsubstanz entstehen Vakuolen, wodurch sich Systeme bilden, deren Aussenschicht eine starke Adsorption für Metalle zeigt. Dieselbe Aussenschicht adsorbiert das Vitamin C und schleusst es durch zur Vakuole (Internum), in welcher der eigentliche chemische Bau der spezifischen Produkte stattfindet. Dabei spielt das Vitamin C

eine Rolle und wird normaliter hierbei verbraucht. Während des Entstehens des Produktes verschwindet der Rest der Golgi-Substanz oder kann wieder als neue Präsubstanz gebraucht werden. Die Hypothese von Ries, dass die Stoffe aus sogen. „Lipochondrien" entstünden, ist unrichtig; die von ihm im Pankreas beschriebenen Körper sind nur Alterspigmente.

3. J. W. Sluiter (Utrecht). Die Cytologie des Hühnereies, während der ersten Phasen der Ovogenese.

Die Ovogenese bei Vögeln dauert sehr lange und ist sehr kompliziert. In diesem Vortrage wurde nur derjenige Teil der Ovogenese besprochen, in welchem noch keine eigentliche Dotterbildung im Ei stattfindet. Die Frage war u.a.: welche funktionelle Bedeutung haben bestimmte sehr kleine Zellorganellen (z.B. Golgi-Körper, Mitochondrien) für den Stoffwechsel der wachsenden Eizelle? Um dies zu untersuchen wurde die von G. C. Hirsch eingeführte Methode der „Stufenuntersuchung" verwendet. Eine grosse Anzahl dicht aufeinander folgender Entwicklungstadien der jungen Eizelle wurde vital und auch auf Schnitten genau untersucht, gezeichnet und zu einer filmartigen Reihe vereinigt. Die Reihenfolge dieser Stadien ergab sich u.a. aus der Kernstruktur, welche besonders im Anfang der Ovogenese eine sehr typische Reihe von Veränderungen zeigt. Es wurden dann mit Hilfe von Lichtbildern einige Ergebnisse dieser Untersuchung gezeigt: im Gegensatz zu den bisherigen Behauptungen, dass die Mitochondrien durch aufschwellen übergehen würden in Dotterkugeln, wurde gezeigt, dass diese Organellen immer dieselbe Form behalten während der Ovogenese. Es ist möglich, dass sie beim Aufbau bestimmter Dottereiweisse beteiligt sind, z.B. als Träger von Fermenten; aber mit den üblichen Farbtechniken kann man diese Funktion der Mitochondrien nicht untersuchen. Die Golgi-Körper aber zeigen eine deutliche Reihe von Veränderungen ihrer Form und ihres chemischen Aufbaues, welche sehr gut übereinstimmt mit der Theorie von G. C. Hirsch über Form- und Stoffwechsel der Golgi-Körper: aus Praesubstanzen entstehen durch innere Vakuolenbildung Golgi-Systeme, in deren Interna ein bestimmtes Produkt aufgebaut wird. Während der Ovogenese rollt eine solche Golgi-Phase zweimal ab. In der Eizelle liefern die Golgi-Körper viele Produkte in Form von Fettkügelchen, welche später bei der endgültigen Dotterbildung verarbeitet werden.

Séance du 20 avril 1940 à Utrecht

1. F. P. KOUMANS (Leyde). Some results of my scientific trip in 1938.

During the preparatory work for the volume on the Gobioidea in M. WEBER & L. F. DE BEAUFORT: The Fishes of the Indo-Australian Archipelago, the characters of several described species were not clear to me. In order to study these species in 1938 a visit was made to musea and other institutions in the United States of N. America, Honolulu, Australia, Netherlands Indies, Philippines and British India. This trip was made possible by a grant of the Pieter Langerhuizen Fonds.

The examination of 294 types of described species showed that 165 names were synonyms of those of already described species. 62 species had to be removed to another genus as the authors did not pay attention to the limits of the older genera, e.g. those of BLEEKER. Several authors published upon specimens of the same species under different names.

The characters of the type specimens several times proved to differ from the characters stated in the description, showing that the species was identical with an already described one.

In the surroundings of Batavia several interesting specimens were collected. Among these there were 10 species not yet recorded from Java, one species was not yet recorded from the North coast of Java.

2. P. D. NIEUWKOOP (Utrecht). L'origine des gonocytes chez les Amphibiens.

A la suite des recherches de M. BOUNOURE concernant la première constatation des futures cellules reproductrices des Amphibiens, j'ai pu faire des expériences microscopiques et expérimentales sous la direction des Messrs. RAVEN et HIRSCH.

Les expériences ont été faites sur des oeufs de *Triton taeniatus*.

M. BOUNOURE croit avoir démontré chez la blastula de *Rana temporaria* des futures cellules reproductrices dans le plancher du blastocoele. J'ai greffé ces cellules au stade de la jeune gastrula d'un animal sur un autre. Les embryons ayant atteints une certaine phase de développement[1]), les cellules reproductrices ont été comptées. Parmi les donneurs se trouvaient des animaux presque stériles, dont le développement était autrement tout à fait normal. Les hôtes avaient à peu près le même nombre de

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[1]) Phase 45 der Normentafel, L. GLAESNER, 1925.

cellules reproductrices que les contrôles. Il est donc possible, que les blastomères du plancher du blastocoele fournissent les futures cellules reproductrices. Il est clair, que les résultats obtenus avec les hôtes ne prouvent rien, parce qu'on n'a pas contrôlé, le comportement du greffon. J'ai l'intention de compléter ces premières expériences et de déterminer au moyen de greffes xénoplastiques le moment, où on peut démontrer définitivement les cellules reproductrices. En 1934 KASEMIERZ LEMBRAT a obtenu de pareils résultats avec *Amblystoma mexicanum* et *Triton alpestris*, mais cet auteur a seulement enlevé, pas greffé l'ébauche des gonocytes.

3.   H. ENGEL (Amsterdam). Anciens cabinets d'histoire naturelle. Publié avec une conférence sur les anciennes ménageries dans ,,De Natuur'', nov.-déc. 1940

Séance du 26 octobre 1940 à Amsterdam

1.   K. W. DAMMERMAN (Leyde). The new fauna of Krakatau.

The investigation into the new fauna of Krakatau has brought us up against the following problems:

1.   The question whether this fauna was totally destroyed in the eruption of 1883.

2.   How long it will last before the islands shall have regained a normal fauna.

3.   The sequence in which the different species of animals have established themselves, and in which way they have reached the islands.

4.   The possibility of the origin of new forms or subspecies. The answer to these questions must remain incomplete in many instances, the faunistic investigation, this in distinction to the botanical researches, having been started much too late. JACOBSON, being the first investigator who made a more complete survey of the fauna, did it in 1908, 25 years after the catastrophe.

JACOBSON as well as the speaker have expressed the opinion that the fauna at least must have been totally annihilated in the eruption of 1883.

As the fauna of Krakatau before 1883 is practically unknown the fauna of some other islands was studied. By comparising these fauna's and that of Krakatau it is probable that Krakatau has already regained 60 to 70 per cent. of its normal fauna after 50 years.

In consequence of the late setting in of the faunistic researches the author could make a study only of the transition of the fauna of the grass wilderness into the fauna of the forest.

The greater part of the new fauna reached the islands through the air, either actively on the wing or spread by the wind. A small percentage only has arrived by sea, carried on driftwood or other flotsam, or by the agency of men or other animals.

The sequence in which the animals have repopulated Krakatau is: first chiefly scavengers, then plant-feeders and only later on predaceous and parasitic species.

The possibility that new forms or subspecies will arise in the islands is indicated by forms arriving from abroad and meeting here different conditions of life. Secondly two forms of one and the same species may reach one of these islands and a new form may originate by hybridisation. Speaker points out the fact that examples of both nature are represented on Krakatau, but deviating forms are not yet recorded, the period of investigation being probably still too short.

The author urges the necessity of continuing the researches and of paying special attention to the repopulation of the new volcano Anak Krakatau recently arised from the sea in the middle of the islands of the Krakatau group.

2. H. J. Vonk und J. van der Krogt (Utrecht). Die Beziehungen zwischen Verdauung und Säuregrad im Säugetiermagen.

Da der p_H des Magensaftes etwa 2 beträgt und das Wirkungsoptimum des Pepsins 2 ist, wurde bisher angenommen, dass das Pepsin im Mageninhalt deshalb unter optimalen Bedingungen wirkt. Diese Vorstellung ist aber zu einfach, erstens weil das Futter im Magen geschichtet liegen bleibt (Grützner) und zweitens weil der Mageninhalt nach Austritt aus dem Pylorus einen p_H von 3 bis 6 zeigt, wobei Pepsin fast unwirksam ist. Es ergab sich nun, dass bei Karnivoren nur die äusserste Schicht des Mageninhaltes einen günstigen p_H annimmt. Diese Schicht wird verdaut, die Produkte werden abgeführt und sodann bekommt eine neue Schicht den günstigen p_H (Messungen mit der Glaselektrode; A. Mennega, Dissertation Utrecht 1938).

Wenn bei Pflanzenfressern nur die äusserste Schicht einen niedrigen p_H annehmen würde, könnte die Eiweissverdauung nur sehr oberflächlich sein, da die Schicht nicht aufgelöst wird. Ausserdem ist zu erwarten dass die Pufferung der Säure durch die Art des Futters hier viel geringer sein wird als bei Fleisch

und dass die Säure schneller eindringt. Wir benutzten als Versuchstier das Kaninchen. Tatsächlich hat sich einige Stunden nach der Mahlzeit die Säure durch den ganzen Mageninhalt verbreitet und weist dieser gleichmässig einen p_H von 1.5-1.7 auf. Überdies ergibt sich, dass auch das Pepsin gleichmässig durch den Mageninhalt verbreitet ist (Extraktion abgewogener Mengen des Inhaltes an verschiedenen Stellen). Dieser Pepsingehalt ist ziemlich hoch.

Die Weise, in der das Futter im Magen den für die Pepsinverdauung notwendigen Säuregrad annimmt, ist also bei Herbivoren und Karnivoren sehr verschieden. Die Omnivoren müssen in dieser Beziehung noch untersucht werden.

3. G. STIASNY (Leyde). Bericht über meine Untersuchungen an der Zool. Station zu Neapel.

Während meines Aufenthaltes an der Zoologischen Station in Neapel im Frühjahr 1940 habe ich zwei verschiedene Untersuchungen ausgeführt.

1. Die Fauna der Alcyonarien und Gorgonarien, die seit den 80-er Jahren nicht mehr studiert wurde. Infolge der Einschränkung der Fischerei musste ich mich immer mehr auf die Untersuchung konservierten Materiales beschränken, das sich jedoch in vorzüglichem Erhaltungszustand befand. In vielen Fällen konnte ich die alten Beschreibungen ergänzen und verbessern, insbesondere was die Bewaffnung der Anthocodia betrifft. *Daniela koreni* und *Cereopsis studeri*, zwei seit mehr als 60 Jahren im Golf nicht wiederbeobachtete und als verschollen geltende Alcyonarien, wurden wiedergefunden und aufs neue beschrieben. Einige für den Golf neue Arten wurden nachgewiesen. Von den meisten Arten wurden neue Habitusbilder gegeben. Im Anschlusse an die Untersuchung der Neapler Formen wurde die Gorgonarien-fauna des ganzen Mittelmeeres überprüft, diejenige des westlichen Beckens mit jener des östlichen verglichen und auf Tethysverbreitung untersucht.

2. Die Entwicklung der Enteropneusten. Seit mehr als 25 Jahren bin ich mit dem Studium davon beschäftigt. Ich konnte endlich einige der mir noch fehlenden Entwicklungsstadien der Metamorphose von *Balanoglossus clavigerus* D. Ch. durch Züchtung (in Seewasser) pelagisch gefischter Larven erzielen. Auch von *Glossobalanus minutus* konnte durch Züchtung (in „Erdschreiber" und Fütterung mit einer Grünalge) die Metamor-

phose der pelagisch gefischten Larve in das benthonische Tier
untersucht werden. Künstliche Befruchtung wurde auf Grund
früherer Erfahrung nicht versucht. Das erste tastbare Resultat
dieser Studien ist, dass nunmehr alle 4 im Plankton des Neapler
Golfes auftretenden Tornarien mit den zugehörigen adulten
Tieren in Verbindung gebracht werden können, was bisher
noch nicht möglich war. Das Material ist noch nicht geschnitten.
Zahlreiche Abbildungen der aufeinander folgenden Entwick-
lungsstadien beider Arten nach dem Leben wurden demon-
striert.

4. S. Dijkgraaf (Groningue). Die physiologische Bedeutung
der Weberschen Knöchel.

Bei den Ostariophysen, die etwa 70% aller Süsswasserfisch-
arten umfassen, findet sich in Form der Weberschen Knöchel
eine mechanische Verbindung zwischen Schwimmblase und
Labyrinth, die geeignet erscheint, Bewegungen der Schwimm-
blasenwand, wie sie bei jeder Druckänderung im Wasser auf-
treten müssen auf die Pars inferior des Labyrinthes zu über-
tragen. Insbesondere der Sacculus ist zur Aufnahme dieser
fortgeleiteten Bewegungen in charakteristischer Weise um-
gebildet.

Durch die Untersuchungen v. Frisch' und seiner Schüler
wissen wir, dass die grosse Hörschärfe der Ostariophysen
auf die Zuleitung von Schwingungen der Schwimmblasen-
wand beruht. Es fragte sich nun, ob auch Schwankungen des
hydrostatischen Druckes, wie sie z.B. bei jeder vertikalen
Bewegung auf den Fisch wirken, auf diesem Wege wahrgenom-
men werden. Bisherige Versuche zu dieser Frage brachten nur
widersprechende Ergebnisse.

Durch Anwendung der Dressurmethode konnte ich zunächst
feststellen, dass die Elritze für hydrostatische Druckschwan-
kungen sehr empfindlich ist: ein Ueber- oder Unterdruck von
$\frac{1}{2}$ – 1 cm Wasserdruck wird noch sehr gut wahrgenommen.
Es zeigte sich nun ferner, dass diese hydrostatische Druck-
empfindlichkeit nach Ausschaltung des Weberschen Apparates
(Exstirpation beider Mallei) völlig verschwunden ist. Sogar ein
Ueberdruck von 40 cm wird nicht mehr wahrgenommen.

Damit ist sichergestellt, dass die Weberschen Knöchel neben
ihrer Bedeutung für den Gehörsinn der Fische auch im Dienste
des hydrostatischen Drucksinnes stehen.

5. L. D. BRONGERSMA (Leyde). Age Variations in the Skulls of Crocodiles.

MOOK, L. MÜLLER and KÄLIN have shown that with proceeding age the relative breadth of the skull increases. Indices showing the relation between length and breadth of the skull in *Crocodylus porosus* Schn. were calculated from the measurements of about seventy skulls. The increase of the relative breadth is found to be the same at different points of the skull. These indices were compared to those calculated from smaller series of *Crocodylus palustris* Less. and *Crocodylus siamensis* Schn. Specific differences are slight, and some importance may be attached to them only if skulls of equal size are compared. The values calculated for *C. siamensis* are generally higher than those calculated for *C. porosus;* those for *C. palustris* again higher than for *C. siamensis*. Individual variation is rather great as the values for two skulls of equal length may differ 5%, for two skulls differing 1 mm in median length, the difference between the indices sometimes amounts to 8%. Specific differences are more evident in an index showing the relation between the length of the snout, and its breadth at its base; the values found increasing in the same direction: *porosus, siamensis, palustris*. An index calculated from the breadth of the palatine fenestra, and the breadth across the narrowest part of the palatina, shows distinct differences between *porosus* (with higher values), and the other two species examined. It is supposed that the great differences found between skulls of about equal size, and of the same species, may be due to differences in sex. Unhappily no record for the sex is given for most of the osteological specimens in the collections used for this study. A skull of *C. siamensis* from Java differs in several respects from skulls from the asiatic mainland; these differences may be due to subspecific differences. The largest skulls measured have a median length of 724 mm in *C. porosus* (Brit. Mus. (N.H.), reg. 47.3.5.33), 526 mm in *C. palustris* (Brit. Mus. (N.H.), reg. 61.4.1.8), and 474 mm for *C. siamensis* (Brit. Mus. (N.H.), reg. 1929. 11.23.1).

Séance du 30 novembre 1940 à Leyde

1. N. VAN TIEL (Utrecht). La régulation du coeur chez *Helix pomatia* L.
Voir Proc. Nederl. Acad. v. Wetensch. 43, 3 (1940).

2. H. Boschma (Leyde). Note on the Rhizocephalan *Loxothylacus variabilis*.

During the Snellius expedition there were collected at Timor a fairly large number of specimens of the crab *Chlorodiella nigra* (Forsk.) infested by a Rhizocephalan parasite. The specimens closely correspond in the peculiarities of the external cuticle of the mantle: this cuticle is covered by a dense layer of small hairs among which there are found a few very long thin spines. By this character the species differs from all other hitherto known Sacculinidae. Internally, however, the specimens show striking differences. In some specimens the characters of the genus *Loxothylacus* appear in a typical manner (distinctly curved testes and visceral mass attached to the mantle at some distance from the stalk), in other specimens these characters are less distinct, whilst in some specimens they are hardly at all apparent. In the latter specimens the testes are almost straight whilst the visceral mass is partly in contact with the region of the stalk. The series of specimens therefore shows a gradual transition from a typical *Loxothylacus* to a form possessing the characters of the genus *Sacculina*. The name *Loxothylacus variabilis* was given to this species to emphasize the particulars given above. More data on this and other species showing corresponding peculiarities are to be found in a paper in Temminckia, vol. 5, pp. 273–372, 1940.

3. Chr. P. Raven – in collaboration with L. H. Bretschneider (Utrecht).
   The effect of centrifugal force upon the eggs of *Limnaea stagnalis*.

Egg-masses of *Limnaea stagnalis* were centrifuged at different stages between the time the eggs are laid and the appearance of the first cleavage furrow. Preliminary observations showed, that eggs centrifuged with a force of 1860× gravity during 5 minutes in many cases developed normally and gave rise to normal little snails. The effect is dependent upon the stage of development, in which the eggs are centrifuged: mortality is highest (nearly 100%), when the eggs are centrifuged between the first and second maturation division (1–2 hours after being laid), much lower before and after this period. The egg substances are separated into three zones: grey yolk (centripetal end), clear protoplasm and yellow yolk (centrifugal end).

This stratification is most distinct, when the eggs are centrifuged just after being laid; the separation of substances is less complete, when centrifuged at later moments. The relative amount of grey and yellow substances is dependent upon the age of the eggs at centrifuging; the light grey materials increase from the time of laying until the first cleavage division, the heavy yellow materials decrease. Normal and centrifuged eggs were treated with various vital stains; moreover, the position of various substances was determined histochemically. The grey zone consists mostly of fatty materials. In the centrifuged eggs the egg substances (glutathione, enzymes) occupy definite positions with respect to the axis of stratification. At further development of the centrifuged eggs the stratification becomes less distinct; the various egg substances tend to spread equally through the egg.

Séance du 1 février 1941 à Amsterdam

H. Boschma (Leyde). Some results of scientific research in the Rijksmuseum van Natuurlijke Historie.

Like anatomists and physiologists, the systematists study one aspect of nature, the principle of their work concerns the study of species and how these may be defined. Besides a thorough examination of the characters of his material the systematist has to study the whole of the literature concerning his group of animals, from 1758, the date of publication of the tenth edition of Linnaeus' Systema Naturae, till now, and, moreover, in many cases he has to examine the literature previous to 1758 for an exact interpretation of Linnaeus' statements. As in other branches of science in systematic zoology there appear, next to numerous papers of real scientific value, a multitude of publications with ill defined data which in themselves form but poor contributions to science. Whereas now in anatomical and physiological researches one can more or less neglect the study of papers of second rate importance, in systematic zoology the study of these papers occupies a great deal of time and energy. In a good paper the author clearly defines his results, so that later investigators know at once what was meant by him. In bad papers the characters of the species are so vaguely and inaccurately put down that it causes later investigators a great amount of difficulty to prove what the author meant. The necessity of preserving type specimens is largely due to the neglect (involuntary, of course) of numerous authors to state the real

specific characters of their material with sufficient accuracy. Unfortunately the lack of distinct descriptions is a wide-spread bad habit, so that type specimens are invaluable for later research by specialists. The preservation of type specimens and other material of documentary value therefore is one of the foremost tasks of the museum systematists. Moreover the material accumulated in a museum in the course of time gives ample opportunity for scientific research. A few examples of results obtained in recent years in the Rijksmuseum van Natuurlijke Historie are given below.

Before 1919 the museum possessed an unsignificant collection of Scyphomedusae and Gorgonaria. Since Dr. STIASNY in 1919 joined the staff of the museum the material of these groups has considerably increased as a result of his investigations. As one of the most striking results of Dr. STIASNY's researches his studies on the canal system of Scyphomedusae may be mentioned, resulting into a better insight into the interrelationships within this group.

A revised catalogue of the collection of Mollusks of the museum, by Dr. BAYER, is in course of publication. In this catalogue, in which, moreover, the species not present in the museum collections are enumerated, the nomenclatorial status of each species is given. This work takes a large amount of labour as in many cases it necessitates an intensive study of the pre-Linnean literature, the interpretation of which not always is an easy task.

Since about a hundred years in the museum the collections of insects have enormously increased, the scientific staff, however, up to the present time always remained too small to complete the necessary investigations. Consequently the scientific research in many groups of insects necessarily has been neglected for many years. An example form the Hemiptera of the museum in which for a period of about 50 years hardly any investigations took place. In recent years Dr. BLÖTE has turned his attention to this group, so that gradually it develops into a perfect museum collection with numerous types and otherwise interesting species. This work necessitates the study of type specimens in museums abroad, as numerous descriptions of previous authors are altogether insufficient for an accurate identification of the species.

To a certain degree the same applies to a family of fishes, the Gobiidae. In this group numerous species have been de-

scribed in an altogether unsatisfactory manner as far as concerns their real characters, whilst in the museum the collections abound in wrongly identified specimens, causing a profound confusion in the literature on this group. In 1931 Dr. KOUMANS published a revision of genera of Gobiidae, afterwards he had the opportunity to examine types and other specimens of the group in numerous institutions in the United States, Honolulu, Australia, the Philippine Islands, Singapore, Java and India. Here he obtained the nacessary data for a monograph of the group which will appear in the near future.

An interesting discovery was made by Dr. KOUMANS in *Oxyeleotris fimbriatus*. Here the rows of scales are not constant in number as in other fishes, but during growth between the existing scales there appear new ones, causing an irregular increase of the number of scales and consequently of the scale rows.

As in other groups of vertebrates in recent years the study of subspecies in Reptiles has yielded interesting results. These are, e.g., very distinct in the snake *Boiga dendrophila* in the western half of the East Indian Archipelago, the Philippine Islands and the Malay Peninsula. Dr. BRONGERSMA found that besides two already previously described subspecies a number of forms of this snake, some of which had been described as varieties, undoubtedly have subspecific value.

Another investigation which could succeed only after the study of an extensive material is that by Dr. BRONGERSMA on variability in crocodiles. There is a large amount of variation among crocodiles of different sizes and probably there are corresponding differences in the two sexes.

Unexpected and striking results were obtained by Drs. JUNGE and VAN DEINSE in their studies on remains of a subfossil whale from localities on the Netherlands coast. These remains could be identified as *Eschrichtius robustus*, till now known from localities in Sweden and England. Moreover they could prove that the subfossil remains are identical with *Rhachianectes glaucus*, the California gray whale, which consequently in prehistoric times occurred in the Atlantic too. Even in historic times this whale must have inhabited the Atlantic, for Drs. JUNGE and VAN DEINSE could show that DUDLEY's scrag whale (*Balaena gibbosa* Erxl.) in reality is the California gray whale. The name of the latter accordingly must be changed into *Eschrichtius gibbosus* (Erxl.).

Since a couple of years the fossils collected in the East Indian Archipelago towards the end of the nineteenth century by Prof. DUBOIS forms a part of the museum collections. These fossils are from comparatively young geological strata and therefore their study necessitates a comparison with recent material. Some years ago Dr. BRONGERSMA made an intensive study of recent Felidae in connection with investigations on the fossil material of the group from DUBOIS' collection. He could prove that four previously described species correspond specifically with the recent tiger, whilst the species *Felis microgale* Dubois is a synonym of *Prionailurus bengalensis* (Kerr). Moreover these investigations yielded interesting results on various species and subspecies of recent cats.

The results mentioned above are a few of the more striking data obtained by the scientific staff of the museum in recent years, for want of time no more particulars could be dealt with in this lecture.

Séance du 8 mars 1941 à Utrecht

1. W. G. WALTER (Amsterdam). Experiments sur l'activité électrique du cerveau.

Voir: CATE, J. TEN, W. G. WALTER and L. J. KOOPMAN. Arch. Néerl. Physiol. 24, 153 (1939); 24, 578 (1939); 25, 27 (1940); 25, 51 (1940).

CATE. J. TEN, and W. G. WALTER. Psychiatr. Neurol. Bl. Sous presse.

CATE. J. TEN, W. G. WALTER and L. J. KOOPMAN. Arch. Neerl. Physiol. 25; sous presse.

2. J. DE WILDE (Amsterdam). Sur la physiologie de l'organe de Johnston et le pouvoir d'éviter des obstacles dans le *Gyrinus*.

Voir Arch. Néerl. de Physiol. 26, (1941).

3. L. H. BRETSCHNEIDER (Utrecht). Das Corpus luteum der Kaltblütler.

Während wit heute über Wesen und Bedeutung des Corpus luteum der Säuger ziemlich genau unterrichtet sind, unterblieb eine dementsprechende Untersuchung bei Kaltblütlern bisher, weil noch stets die Meinung gilt, dass Anamnier kein Corp. lut. besitzen. BRETSCHNEIDER und DUYVENÉ DE WIT haben vor eini-

gen Jahren beim Bitterling (*Rhodeus amarus*) im Ovarium endo-
krine Drüsen entdeckt, die wie das Corp. lut. der Säuger aus
dem Eifollikel enstehen und einerseits mit der Hypophyse,
andererseits mit dem bekannten Legeröhrenwachstum zusam-
menhängen. Es ergab sich, dass in diesen Drüsen ein Hormon
gebildet wird, welches die Legeröhren-Verlängerung bewirkt;
wir nannten es „Oviductin". Aus diesem causalen Zusammen-
hang zwischen: Hypophyse (gonadotropes Hormon) bewirkt im
Ovarium die Bildung von endokrinen Drüsen aus Eifollikeln und
deren Produkt Oviductin, welches auf die Legeröhre einwirkt,
haben wir geschlossen, dass diese Drüsen Corpora lutea sind.
Es ergab sich die Frage, ob auch bei anderen Kaltblütlern diese
Corpora lutea vorkommen. Die Untersuchung einer grösseren
Reihe von Anamniern und Sauropsiden zeigte, dass das Corpus
luteum ein intergrierender Bestandteil des Ovariums aller
Vertebraten ist. Dabei ergaben sich zwei Typen von Gelb-
körpern: 1. bei den Anamniern entsteht das Corp. lut. bevor
der Follikel ovuliert, und hängt zusammen mit einer gleich-
zeitigen Resorption des Eies durch das Follikelepithel, weshalb
wir diesen Typus das Praeovulations C. l. nannten; 2. bei
den Sauropsiden und Mammaliern entsteht das C. l. erst nach
der Ovulation des Follikels, weshalb wir es das Postovulations
C. l. nannten; bei den Reptilien kommen beide Typen in
ungefähr gleicher Anzahl vor. Nebst der morphologischen
Identifikation dieser C. l. haben wir auch bei *Rhodeus amarus*,
Lophius piscatorius, *Lebistes reticulatus* und *Zoarces viviparus* physio-
logische und endokrin-experimentelle Beweise zu erbringen
versucht und damit nachgewiesen, dass die praeovulations
Corp. lut. der Kaltblütler mit Recht die Bezeichnung Corpus
luteum verdienen. Mit diesen Feststellungen ist der Biologie
ein neues Untersuchungsfeld erschlossen.

Séance du 3 mai 1941 à Utrecht

B. J. KRIJGSMAN (Utrecht), Bericht über und Demonstrationen
   mit dem Kathodestrahloszillograph.

Es wird betont, dass die Elektrophysiologie zur exakten
Untersuchung von bioelektrischen Prozessen Messinstrumente
braucht, welche dermassen empfindlich sind, dass sie noch
Potentialschwankungen von etwa 10 μ V anzeigen können und
dermassen geschwind, dass sie den Verlauf schneller Spannungs-
fluktuationen fehlerlos aufzeichnen. Verschiedene Apparate,

welche man im Laufe der Zeit zu diesem Zweck benutzt hat, werden kurz besprochen. Die technischen Fehler, welche den elektrophysiologischen Untersuchungen durch Anwendung ungeeigneter Instrumente anhafteten, wurden mit einem Schlag aufgehoben durch die Einführung des Kathodestrahl-oszillographen (GASSER und ERLANGER 1922). Die Grundlagen der Kathodestrahloszillographie werden erörtert und darauf hingewiesen, dass man mit Hilfe von horizontalen Ablenkplatten das Phänomen zweidimensional auf dem Schirm der Röhre erscheinen lassen kann. Schliesslich wird ein Elektrokardiogramm, Aktionsströme von Skeletmuskeln sowie Aktionsströme vom N. ischiadicus eines Frosches vorgeführt.
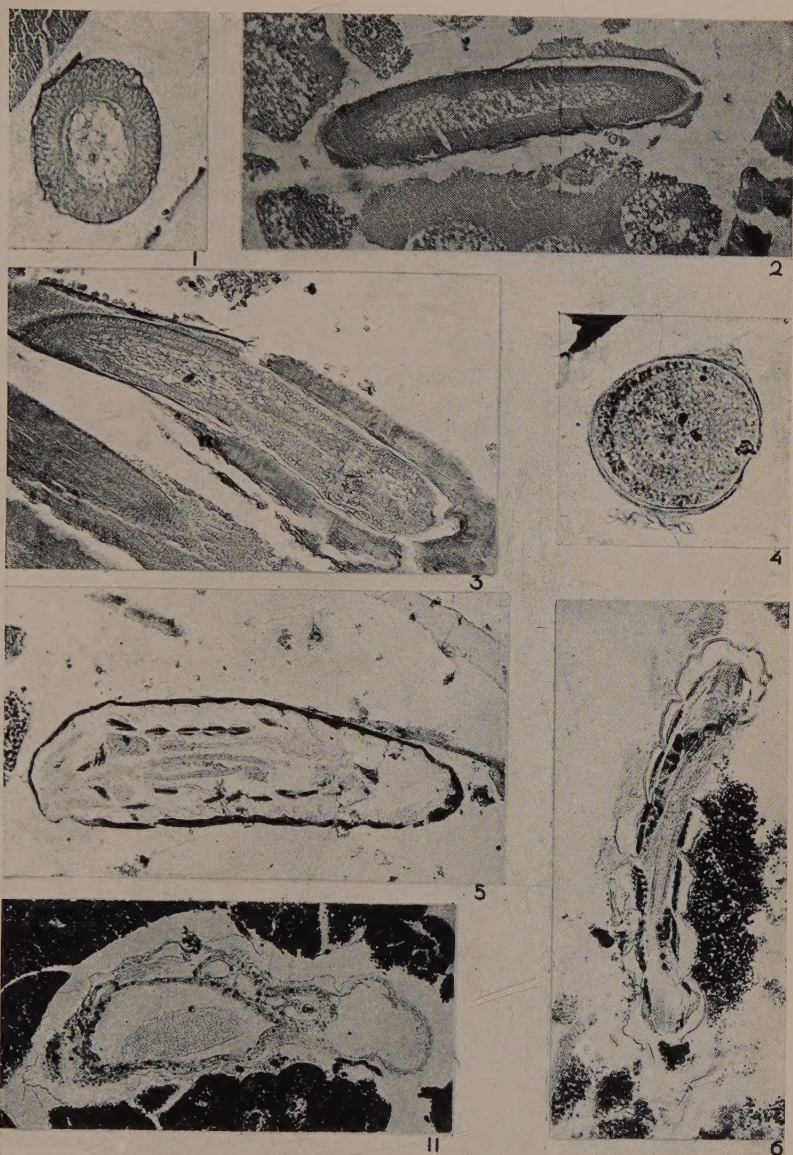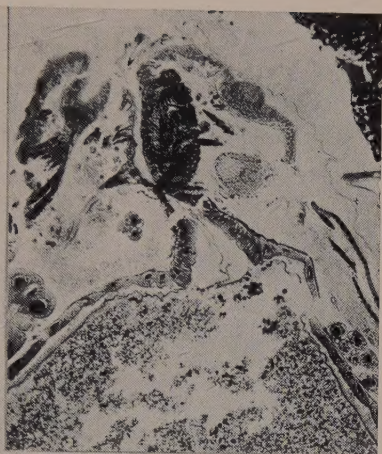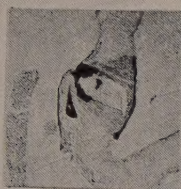
Photos 1 to 6 and 11

L. W. D. CAUDRI.   The Braconid *Alysia manducator* Panzer in
its relation to the blow-fly *Calliphora erythrocephala* Meigen
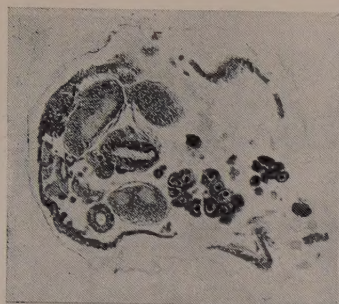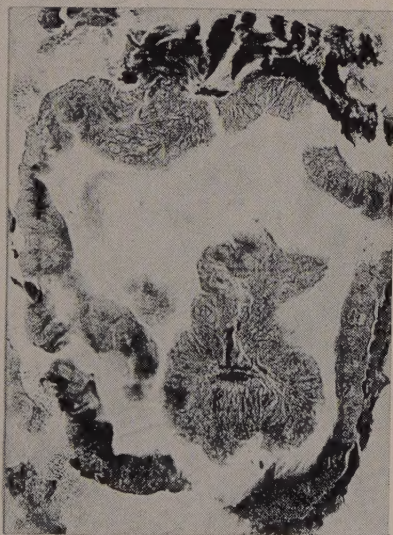
Photos 7 to 10 and 12

L. W. D. Caudri. The Braconid *Alysia manducator* Panzer in its relation to the blow-fly *Calliphora erythrocephala* Meigen